THE LIFE–HISTORY OF *GREVILLEA ROBUSTA* (Cunn.).

By P. Brough, M.A., B.Sc., B.Sc.Agr., Lecturer in Botany, University of Sydney.

(Plate ii; ninety Text-figures.)

[Read 29th March, 1933.]

Introduction.

More than thirty years ago Professor D. H. Campbell (1897, pp. 2 and 3) stressed the fact that the classification of the Angiosperms was little more than mere guess-work, and in considering a remedy for such a regrettable state of affairs remarked: "How greatly would the value of many an important monograph on a genus or family be enhanced did it but include a connected account of the whole life-history of one or two representative forms"; and again, "It is true that the structure of the flower and fruit of the Angiosperms is of the greatest importance in their classification, but these alone are not sufficient for settling positively questions of affinity, except between nearly related groups. An accurate knowledge of the development and histology of the reproductive parts and embryo is also very important in this connection. Not until very much more is known than at present about the life-history of representatives of all the principal types of flowering plants, shall we be in a position to begin to build up a system of classification which we can hope to be even approximately accurate."

It is significant to observe that almost twenty years later—in 1916—Professor Pearson (1929, p. 179) in making a morphological comparison of the embryo-sac and endosperm of *Gnetum* with those of Angiosperms bewailed the fact that "Even now, the number of Angiosperm sacs investigated is comparatively small." Again, recently Rendle (1925, p. 424) makes the following observation: "The consideration of the sympetalous Orders together after the dialypetalous is largely a matter of convenience and must not be regarded as implying that they form a distinct natural class."

Complaint then regarding the paucity of information on the details of life-histories of types representative of the whole Angiosperm phylum is even at the present day widespread, and the call for further research is clearly insistent.

While some cry out upon the monotonous regularity of the development of the reproductive parts in Angiosperms, others again are seized with the idea that therein lies the most valuable approach to the solution of certain problems of outstanding phylogenetic and systematic importance. Adherents of the latter view then, need never look beyond such a field for problems worthy of the most zealous investigation.

The writer, convinced of the real value of any additions to our knowledge of the several phases of the Angiosperm life-history, has endeavoured during the last few years to throw some further light on this subject by investigating forms which are almost confined to Australia (Brough, 1923; 1924; 1927).

Newman (1928, 1929) has also made valuable contributions along similar lines, and it may be suggested that, with further additions of a like nature, means may be placed at our disposal, which will permit of the elucidation of such major problems as the Origin of the Australian Flora, the spread of the

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Angiosperms in general, and the real relationships existing between the Families of flowering plants.

This present publication embodies the results of studies in the Proteaceae, a family which has received much attention—particularly in Australia—from many investigators specially interested in xerophytes, and their xeromorphic and edaphic adaptations. This family has been markedly neglected by those engaged in the investigation of Angiosperm life-histories (except by Ballantine, 1909).

Bews (March, 1927, pp. 6 and 7) furnishes an account of the past and present distribution of the Family, and draws attention to the diverse views held by such eminent palaeobotanists as Scott and E. W. Berry regarding its fossil history and the interpretation thereof.

The Proteaceae comprise 55 genera and some one thousand species (Bews, May, 1927, p. 69), and a study of the more outstanding systems of classification shows how incompatible are the views held by past and present-day systematists. In this connection Bews observes: "According to Engler's arrangement the Proteales connect with the Santalales: Hutchinson thinks they are allied with the Thymeliaceae, Balfour was of the opinion that they had affinities with the Roseales." Further, Rendle (1925, p. 58), dealing with the Santalales and Proteales, remarks: "While undoubtedly allied to each other, it is difficult to associate these Orders with other groups."

The obvious deduction from such a number of conflicting opinions is that the present data are insufficient to settle the questions under dispute. Accordingly the need for the exhaustive study of the life-history of at least one Australian representative of the Family need no longer be stressed.

The Proteaceae and the Problem.

The Proteaceae form a very conspicuous component of, and in many regions impart a distinctive facies to, the Australian flora, which embraces some twenty-nine of the fifty genera and about 575 of the one thousand species, included in this large Family (Bentham, 1870). Sargent (1930, pp. 577 and 578) points out that the Australian representatives are massed chiefly in Western Australia, "Seeing that it possesses nearly twice as many species as all the other States of the Commonwealth together". He also supplies the following interesting comment: "It seems, therefore, quite beyond dispute that this Family, whose members are generally regarded as typical xerophytes—drought resisting plants, often, indeed, drought loving plants—as a Family has a strong preference for the moister regions of our Island Continent."

Accordingly it is not surprising to find the Proteaceae well represented in New South Wales, where some 17 genera and 135 species are located (Moore and Betche, 1893) amid an environment which cannot be regarded as xerophytic (Osborn, 1932).

Abundant material for an investigation of the nature proposed is therefore easily available, and an early consideration was to decide which genus and species most readily lent itself to the desired objectives. In making a final selection it was very necessary to keep in mind that the research must primarily concern itself with an endeavour to glean information regarding (a) the nature of the perianth, (b) the significance of the nectar scales common—though in various degrees of size—to members of the Family, (c) the frequent zygomorphy, (d) the morphology of the gynoecium, (e) the origin, development, and structure of the ovule, (f) the gametophytes, (g) pollination, (h) fertilization, (i) embryology, (j) the cause of the pronounced low seed production, and (k) the

morphological nature of the seed-wing. Other features might be expected to arise and to demand elucidation as the work progressed.

It seems reasonable to state that detailed information regarding the points enumerated must decide, or at least very materially aid in determining, the vexed question of the affinities and systematic position of the Family.

GREVILLEA ROBUSTA (General).

Grevillea, the largest of the Protead genera, comprises 160 species, and of these 45 are native in New South Wales.

Only as the result of much preliminary investigation was it decided to make *Grevillea robusta*—the Silky Oak—the material for intensive study. Many other types of this and other genera had been considered, but were in turn rejected, either on account of the early thickening of the ovary wall, or the low percentage of fertilized ovules or both.

Reference to Plate ii will readily indicate the general features of the form chosen, which has the advantage of being readily obtainable, has a highly specialized floral structure, and above all produces a comparatively high percentage of seeds with well developed embryos. This last character assumes enormous importance, when one realizes the notoriously low percentage of seeds set by the very great majority of the various species of the Family, and the consequent great difficulty in tracing the embryology. *Grevillea robusta* is one of the dendroid representatives of the Proteaceae, and may attain to a height of 120 feet, with a stem diameter of about 3 feet (Francis, 1929, p. 83).

Its natural habitat is in the brush forests, where it ranges from the Clarence River in New South Wales to Northern Queensland (Maiden, 1917, p. 175). Being a brush type, its range inland is strictly limited, no specimen having been reported beyond a distance of one hundred miles from the coast.

The Silky Oak, on account of its great ornamental value, has been planted freely in and around Sydney, so that abundant material is to hand. In sheltered aspects of this—the Sydney—district anthesis commences about the beginning of October, and continues until mid-December in the more exposed localities. In full bloom the tree compels attention, not only on account of the brilliant orange-yellow of the densely massed inflorescences, but also because of the abnormal nectar production, which attracts great numbers of bees and numerous nectar-seeking birds, the ensemble being an extremely lively one from the naturalist's point of view.

The inflorescence is a panicle, the individual racemes being three to six inches long, and varying in position from almost horizontal to nearly vertical. The raceme supports numerous flowers, each on a pedicel about half an inch in length (vide photo, Plate ii).

Reference to Text-figure I, 1–27, shows various phases in floral development—the young bud, the looping of the style, its gradual emergence between two perianth segments, the final freeing of the stigmatic region, the recurving, contraction, and fall of the perianth segments, and finally several stages in development of the fruit. It is to be noted that the style bursts through the perianth on the side remote from the axis of the inflorescence, and the tip curves over adaxially. The perianth segments, bearing the dehisced anthers, as a rule, fall within a day or two of anthesis, and subsequently a small ovary mounted on a gynophore, which in turn is supported by the long pedicel, stands exposed. Anther dehiscence extends over the period just prior to, and simultaneous with, the escape of the style, and so pollen is available for distribution from the top

of the expanded style, which bears aloft almost all of the pollen in a cone-shaped mass. The stigmatic surface is not yet exposed, however (Text-fig. I, 10–11).

It is noteworthy that in the early bud condition the angle between the pedicel of the flower, and the peduncle of the raceme is acute, but as anthesis approaches, this angle increases to about 90° and later may become very oblique.
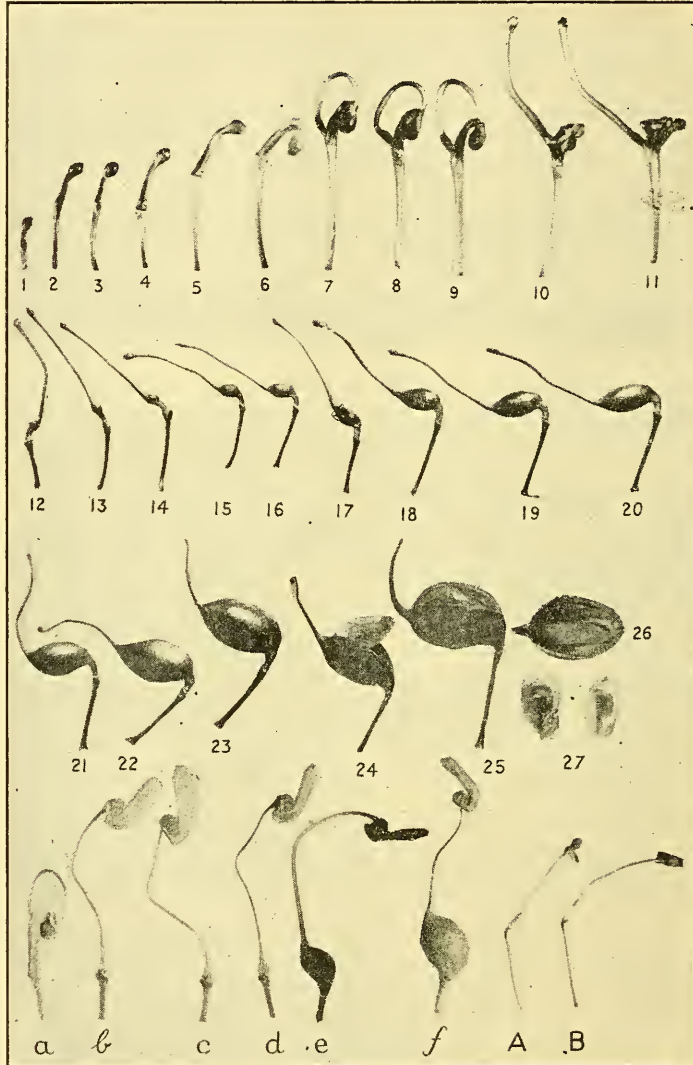
Text-fig. I.
Series 1-27 illustrates the development of the flower-bud, flower, fruit and winged seed of *Grevillea robusta*; the follicular nature of the fruit, and its method of dehiscence are also depicted. × 1.
Series *a-f* shows successive stages in the production of a fruit from a "capped" flower of *Grevillea Banksii*. × ⅔.
Series A and B illustrates fruit production from "capped" flowers of *Grevillea robusta*. × ⅔.

This change obviously places the flowers in more advantageous position for pollination, since birds alight on the stronger basal region of the inflorescence— the distal end being too fragile for their support. The open flowers thus conveniently face the pollinating visitor.

Floral Organogeny.

In order to study the origin and early development of the floral members, transverse and longitudinal microtome serial sections of complete young inflorescences and also of mature individual flowers were examined.

A raceme in longitudinal section, as illustrated in Text-figure 1, consists of an elongated central axis or peduncle on which are arranged, in acropetal order, the numerous floral buds, which, as is clearly demonstrated, arise in pairs and all round the axis (Text-fig. 2), each pair being located in the axil of a single hairy bract (Text-figs. 1, 2, 3 and 5) which is early deciduous. This twinning of the flowers is readily perceived in the genus *Banksia*, even in mature inflorescences, but in *Grevillea robusta* this feature tends later to be obscured owing, in the first place, to the heliotropic twisting of the pedicels and, secondly, to the fall of many young buds. In this form no exception to the paired arrangement of the flower buds was encountered, though numerous serial sections were examined.

The first evidence of the flower is a rounded mass of tissue (Text-fig. 1 at A) on which, as development proceeds, four symmetrically arranged protuberances are recognized. Two of these are evident in Text-figure 1 at B, and such represent the primordia of the perianth segments. A bract subtends each pair of buds (Text-fig. 2).

By following the peduncle basipetally in Text-figure 1 the gradual increase in size of the perianth segments may be traced, and the inception of a second and inner set of four primordia recognized. The illustration in Text-figure 4 shows such primordia at a more advanced stage, and their further development is depicted in Text-figures 4–9 inclusive.

The primordia referred to, which are situated directly in front of and within the perianth segments, are those of the four stamens, each of which becomes epiphyllous at a very early stage of development, because very soon after their inception, the meristematic region of each perianth segment fuses with that of the stamen immediately within (Text-fig. 4), so that the two fused floral members continue growth as a single structure until the mature condition is attained when the anther, supported on, and in conjunction with, a very short part of the filament, is the only free part of the stamen (Text-fig. 9).

It will be noticed (Text-fig. 1, at B) that a vascular strand passes from the stele of the peduncle to each of the subtending bracts. Again, vascular tissue runs to the base of each pair of flower buds, at the base of which it bifurcates, one fork passing to each flower. Each of these bundles then divides into five, one branch passing into each perianth segment and the base of the organic apex respectively (Text-fig. 5), and each of these bundles in turn splits into two (Text-fig. 1, at C) one portion supplying the conjoined stamen while the other proceeds towards the distal end of the perianth segment.

Again, reference to Text-figures 4–9 shows that the primordium of the single carpel makes an early appearance. Its true nature is, however, best illustrated in a series of transverse sections of buds at successively more advanced stages in development (Text-figs. 12–14), where the fact that the gynoecium consists of a single carpel—clearly an infolded simple leaf-like structure—is amply demonstrated.
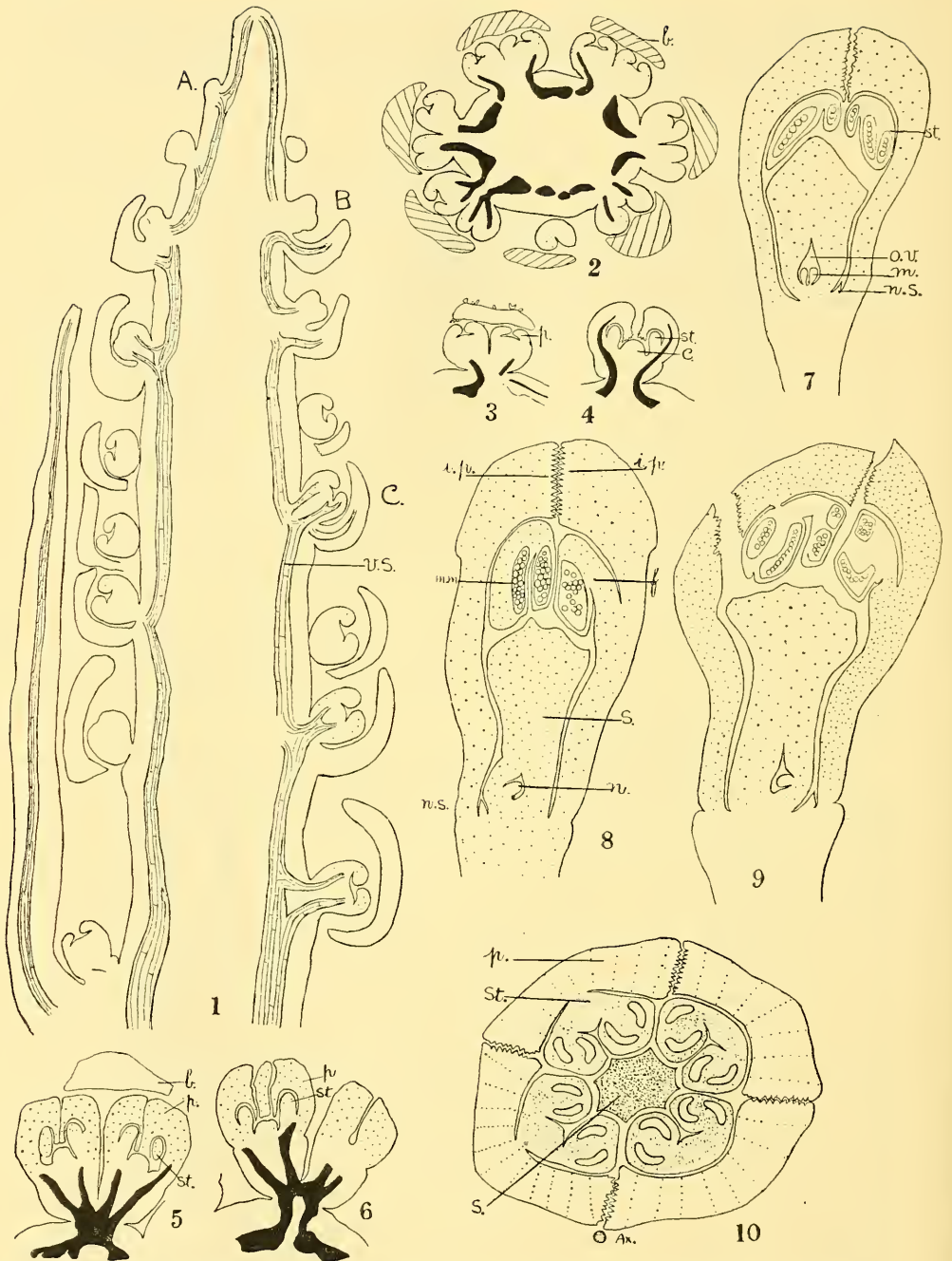
Text-fig. 1*.—A median longitudinal section of a very young inflorescence illustrating various phases in development of the flower bud and subtending bract. The vascular system of the peduncle, *v.s.*, and its continuity with that of the buds and bracts is indicated.   x 35.

At the very young stage depicted in Text-figure 12, a midrib with two lateral branches is evident, and at a slightly older stage two additional bundles make their appearance (Text-fig. 13). Further increase in size of the carpel results in a marked increase in the number of vascular tracts until, at the stage represented in Text-figure 14, as many as 13 bundles may be observed. A marginal bundle passes into the funiculus of each of the young ovules depicted in Text-figures 14 and 26. Also, in Text-figure 12, the incipient marginal placentae which later give rise to the two ovules of the carpel are just evident at X, while in Text-figures 15, 16, 26, 33, 34, 57 and 58, the origin and general structure of the young ovules are shown.

Again, very soon after the gynoecium is initiated, an upgrowth of paren-chymatous cells from the torus is noticeable at the base of the carpel and on the adaxial side. This tissue gradually increases in height, and later forms a conspicuous crescent-shaped nectar scale. It is well developed even in such an early bud stage as is illustrated in Text-figures 7–9.

All the floral organs have thus become established, and it only remains to follow their development until maturity of the flower is attained. The position of each member relative to the axis of the inflorescence will also require careful elucidation.

In order to gain a proper appreciation of what may be regarded as the essential floral structure of *Grevillea robusta*, a series of transverse sections (Text-figs. 17–32) and also longitudinal sections (Text-figs. 33 and 34) at the stage just prior to anthesis were prepared. An appreciation of the general structure of the flower bud, as shown in Text-figure 33, will readily indicate the particular levels at which the various transverse sections of the series were made.

Examination of selected transverse sections taken at intervals and in acropetal order indicates a shallow concavity in the floral receptacle. Thus the flower is slightly perigynous. Additional evidence of this is supplied by a study of longi-tudinal sections of buds at various stages of development (Text-figs. 9, 11, 33 and

Text-fig. 2.—A transverse section of an inflorescence cut about the region C in Text-fig. 1. The twinning of the floral buds with subtending bract, *b.*, and the vascular system are shown. × 35.

Text-fig. 3.—A median longitudinal section of two slightly older buds showing perianth segments, *p.* × 35.

Text-fig. 4.—A median longitudinal section of a still older flower depicting the young anthers, *st.*, and the central upgrowth of carpellary tissue, *C.* × 35.

Text-figs. 5, 6.—Longitudinal sections of twin flower buds slightly more advanced than in Text-fig. 4. A vascular supply from the peduncle to each bud, and a faint lobing of the carpel are shown ; *b.*, bract ; *p.*, perianth segment ; *st.*, stamen. × 35.

Text-fig. 7.—Median longitudinal section of flower-bud cut at right angles to the antero-posterior plane. Microspore mother-cells are present in the anther, *st.* ; two young megasporangia, *m.*, are seen in the ovarian cavity, *o.v.*, and the nectar-scale, *n.s.*, is visible at base of carpel. × 35.

Text-fig. 8.—Median longitudinal section of flower-bud cut in antero-posterior plane. *n.*, nucellus ; *f.*, filament of anther ; *m.m.*, microspore mother-cells ; *n.s.*, nectar-scale ; *i.p.*, interlocking perianth segments ; *s.*, style. × 35.

Text-fig. 9.—Longitudinal section as in previous figure, but at a slightly older stage : The microspore mother-cells are undergoing reduction division. × 35.

Text-fig. 10.—Transverse section through apical region of flower-bud : *p.*, perianth segment with epiphyllous stamen, *st.*, containing four loculi ; *ax.*, axis of inflorescence ; *s.*, style. × 50.

* All drawings, with the exception of that in Text-figure 83, are camera lucida.
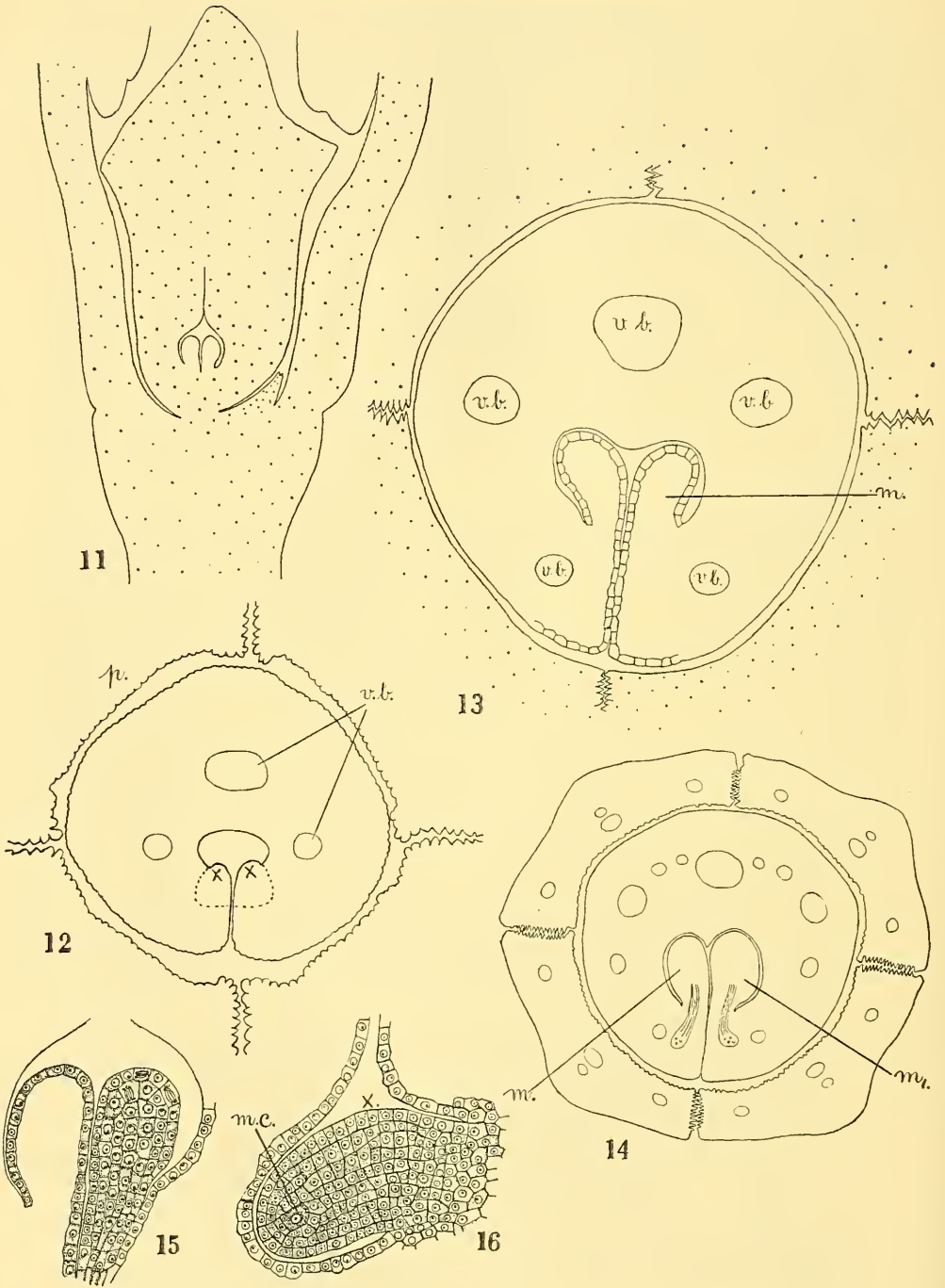
34). From the torus the perianth segments, stamens, nectar-scale and carpel gradually differentiate centripetally. At first the perianth segments are in close contact, but the subsequent extraordinary growth in length of the style necessitates its curvature, which takes place in the antero-posterior plane. With further increase in its length a decided loop is formed which forces all the segments slightly apart, but the gap between the two abaxially placed perianth segments is steadily emphasized, and it is through this aperture that the loop of the style eventually is thrust (Text-fig. 31; also Text-fig. I, 6-9).

The series of transverse sections depicted in Text-figures 17 to 32 illustrates the structure of the flower at various levels. The lobed carpel, the adaxial nectar-scale, the relative position of the separate perianth segments, the ovarian cavity, the marginal position of the young ovules, the style—in part within and in part without the perianth—the fusion of the stamens with the perianth segments, and the cone-shaped nature of the apex of the style are all clearly delineated.

In Text-figure 32 of the series the apical part of the flower is seen in longitudinal section, this of course being due to the curved nature of this region, which is approximately at right angles to the axis of the flower as a whole. At this stage the anther contains tetrahedral pollen grains.

The carpel is so orientated that the line of fusion of the margins is adaxial and adjacent to the central region of the nectar-scale (Text-fig. 22). The position of these organs relative to the perianth segments is also demonstrated.

It is to be noted—despite statements to the contrary—that the primordia of the perianth segments are separate from the first, and that the fused condition does not exist at any stage of their development. None the less it is evident that in the young bud the margins of adjoining perianth segments fit into each other with extraordinary closeness and precision by means of interlocking teeth-like cells (Text-figs. 8, 10 and 35). The rigidity of this structure results in a simulation of the sympetalous condition.

In the young bud condition then, the style keeps pace with the development of the perianth segments, but subsequent restriction in rate of growth of the perianth does not mean a similar slowing down in style elongation, which steadily proceeds. It might be expected that the style should force its way out

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Text-fig. 11.—Median longitudinal section of lower part of a flower-bud cut as in Text-fig. 7, showing carpel, ovarian cavity, two young megasporangia, and nectar-scale. × 225.
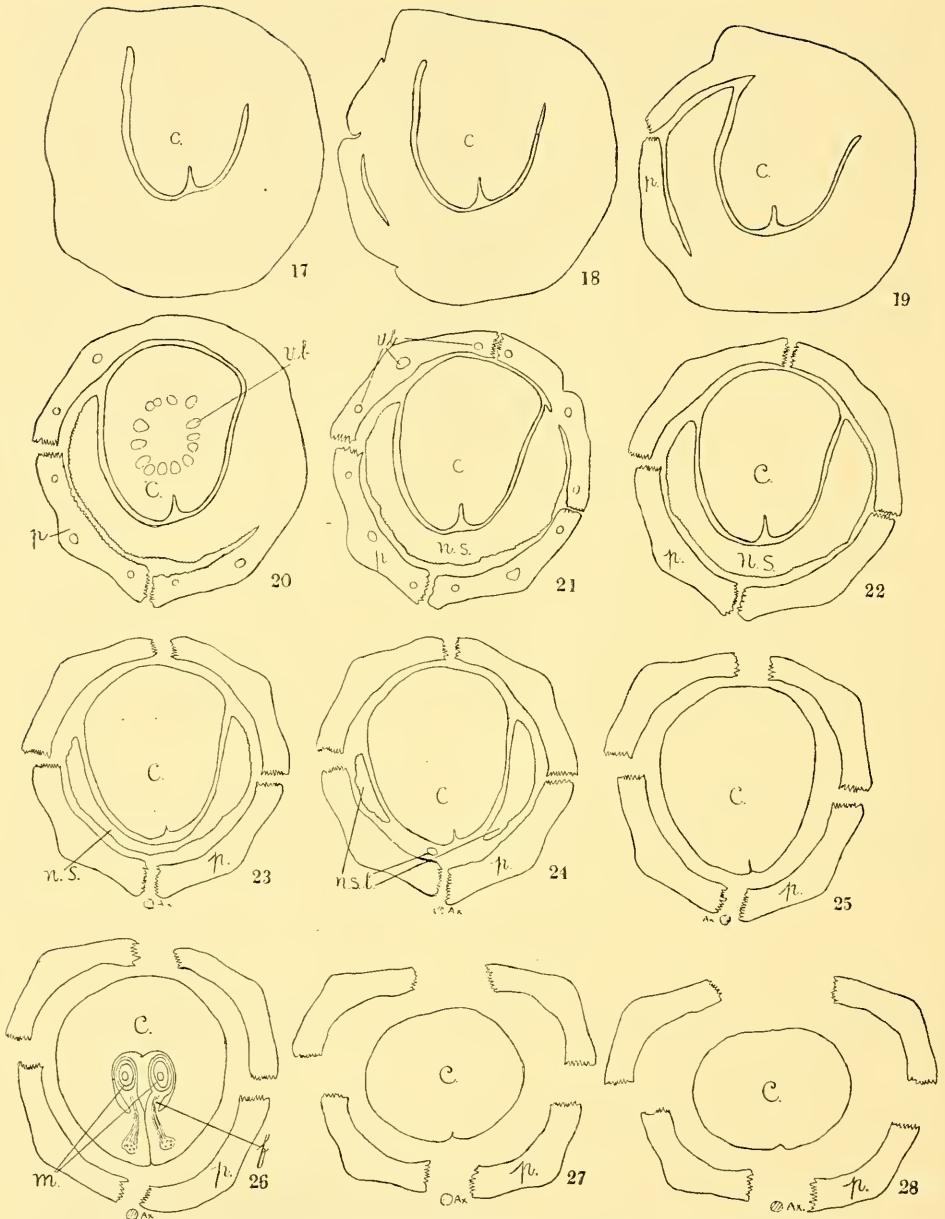
Text-fig. 12.—A transverse section of a young flower-bud showing inner margins of perianth segments, $p.$, enclosing a very young carpel with infolded margins; $v.b.$, vascular bundles; x, meristematic region giving rise to placenta and nucellus. × 135.

Text-fig. 13.—A transverse section of a bud slightly older than in previous figure. The epidermal cells of the infolded margins of the carpel and an increase in the number of its vascular bundles are depicted. $m.$, megasporangium; $v.b.$, vascular bundle. × 135.

Text-fig. 14.—A transverse section of a still older bud showing the vascular bundles of perianth and carpel respectively. The two megasporangia, $m.$ and $m_1.$, seated on the margin of the carpel are evident. A vascular bundle is seen to pass from the margin of the carpel into the chalazal region of each nucellus. × 55.

Text-fig. 15.—Vertical section in lateral plane through the ovarian cavity containing two young megasporangia. × 225.

Text-fig. 16.—Median vertical section in antero-posterior plane through a young nucellus. The origin of the incipient inner integument is indicated at X. The embedded megaspore mother-cell, $m.c.$, is evident. × 225.

Text-fig. 17-32.—A selection of transverse sections taken from a complete acropetal series cut through a flower bud at the stage of development indicated in Text-fig. I, 5. These sections illustrate the general structure and relative positions of the component parts of the flower. Sections 17-21 are slightly oblique to axis of flower. For detailed description consult text. *c.,* carpel; *p.,* perianth segment; *n.s.,* nectar-scale; *n.s.l.,* nectar-scale lobes; *m.,* megasporangia; *f.,* funiculus; *s.,* style; *st.,* stamen; *s.d.,* slit of dehiscence; *m.,* microspores; *Ax.,* axis of raceme. × 25.

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31

32

st.
m.

st.

s.d
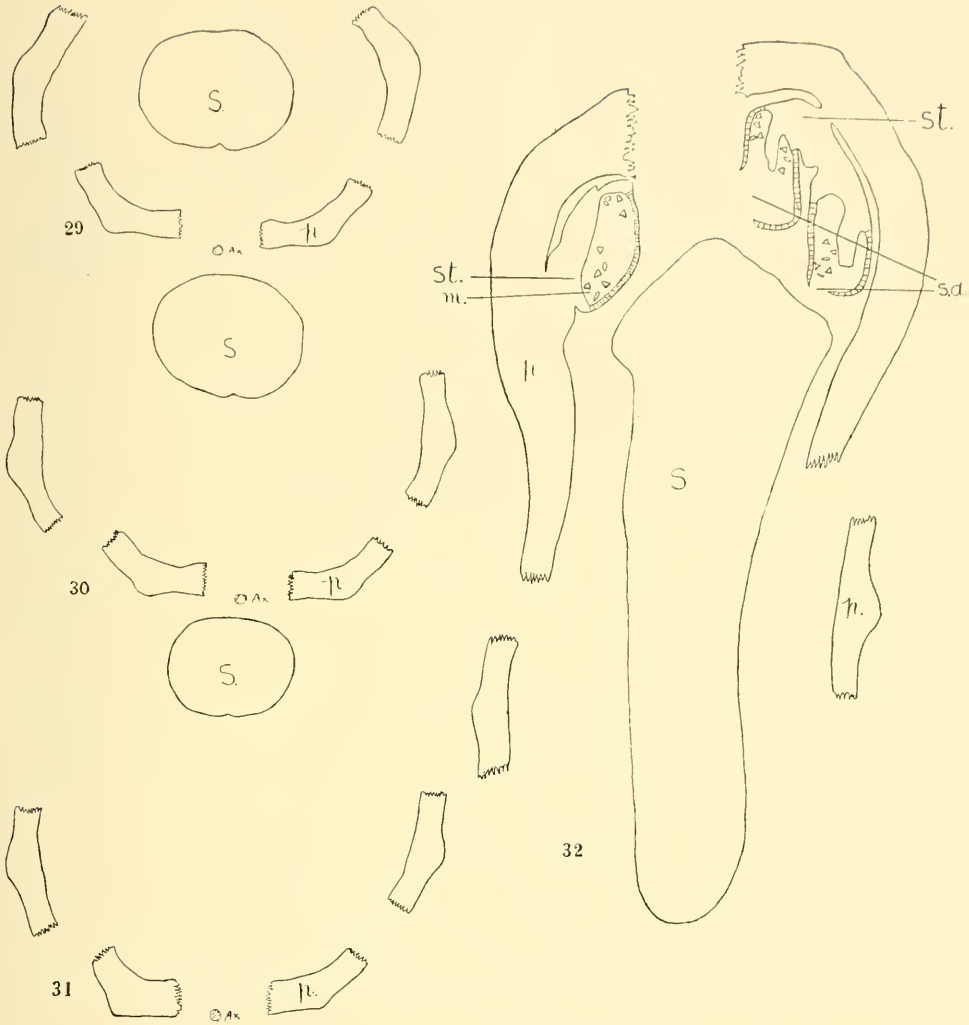
through the perianth segments at the apex of the flower, but in this region, the interlocking of the cells, which is specially marked at the distal ends of the perianth segments, exerts sufficient resistance to overcome the upward thrust, with the result that the middle region of the style curves and eventually forces its way out between the two abaxial segments, where there must be less resistance, even though interlocking occurs in this region also. Examination of the style shows elongation of the cells on the outside of the curve, and compression of those on the convex face. However, this steady increase in length of the style imposes a very considerable and ever increasing strain, with the result that the distal end of the style eventually frees itself by forcing its way through the interlocked ends of the perianth segments, which, thus relieved suddenly from an elongating tension, contract. They then appear as somewhat incon-

spicuous coiled structures at the base of the floral axis (Text-fig. I, 10–11). Thereafter they are soon deciduous—only persisting for a few days.

Investigation of sections cut longitudinally through the flower bud (Text-fig. 8) shows a well-defined absciss layer of cells (Text-fig. 36) just adjoining the base of the perianth.

The line of fracture is visible in Text-figure I, 7–10. Thus, perianth fall in regard to time and tissue concerned is determined with precision beforehand.

Now, although the above description represents the normal behaviour of the flower at this stage of development, it is interesting and significant to record that in some cases the apex of the style does not forcibly free itself by a sundering
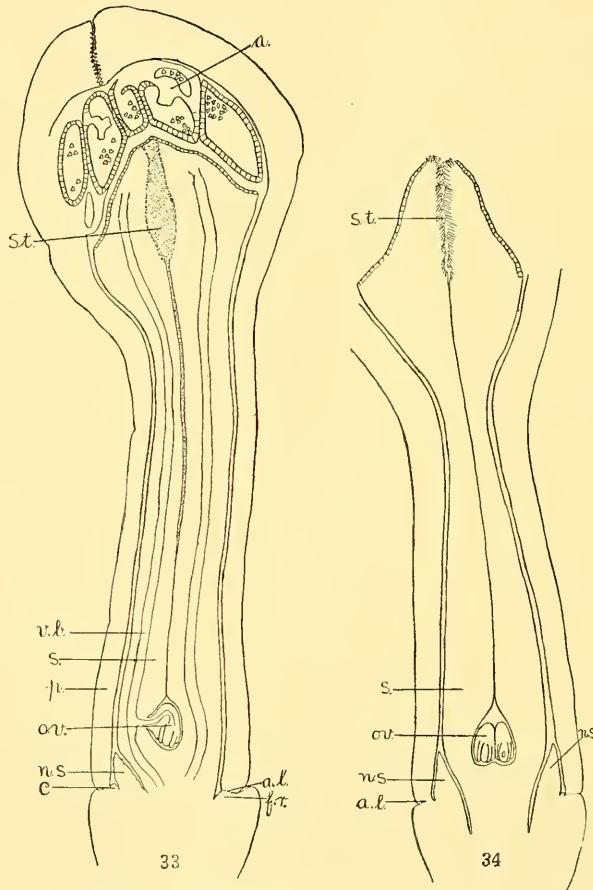
Text-fig. 33.—Median longitudinal section of flower bud at stage 2 of series in Text-fig. I. *f.r.*, floral receptacle; *a.l.*, absciss layer; *n.s.*, nectar-scale; *c.*, nectar-secreting cushion between nectar scale and perianth; *o.v.*, ovule; *p.*, perianth; *s.*, style; *v.b.*, vascular bundle; *s.t.*, stigmatic tissue; *a.*, anther. × 25.

Text-fig. 34.—Median longitudinal section of lower part of a bud slightly older than, and cut at right angles to, that shown in previous figure. Interpretation as in Text-fig. 33. × 25.

of the interlocked distal ends of the perianth segments, but instead, such segments are ruptured at their individual bases, along the absciss layer, and are carried up on top of the style (Text-fig. I, A, B) just as occurs in such well-known cases as the corolla in *Vitis* and the staminate flowers of *Casuarina*. It therefore follows that in these particular cases the resistance exerted by the interlocking at the apex of the perianth segments is great enough to force a prior rupture at the bases of these segments. When this happens, the force tending to sunder the apices of the segments is destroyed, with the result that the perianth becomes lodged as a "cap" on top of the style until long after the pollination period has elapsed. Further discussion regarding this phenomenal behaviour will be deferred until the general question of pollination is dealt with.

In the normal flower anthesis is completed by the sudden disengaging of the distal region of the style from the enclosing perianth segments.

The monocarpellary ovary has a bulbous-shaped hollow ovary supported on a gynophore, and surmounted by a long curved style (Text-fig. I, 12–25). The apex resembles a low cone with a blunt top which, at the moment of liberation, is piled high with pollen. Immediately subsequent to this stage, the cells occupying the central region of the distal end of the style grow rapidly, rupture the surmounting epidermis, and form a loose mass of elongated cells disposed around a funnel-like opening (Text-figs. 33 and 34). This cavity gradually widens and exposes the elongated glandular cells of the stigma (Text-figs. 37, 38 and 39). At the same time this central upgrowth displaces centrifugally any microspores which have not been dispersed, and causes them to be removed from possible contact with the stigmatic cells.

The flower thus shows extreme protandry, and enforces cross pollination. The mature stigmatic tissue, when examined in longitudinal and transverse section respectively, is seen to be composed of long vacuolate cells (Text-fig. 39), which, lining a deep pocket (Text-fig. 37), furnish an ideal medium for the development of the male gametophyte.

Nectar Secretion.

Profuse nectar secretion is a characteristic of the Proteaceae, the flowers of which are provided with nectar-secreting organs of very varying form. When large and separate from one another, as, for example, iu the genus *Banksia*, they are seen to alternate in position with the perianth segments.

In *Grevillea robusta*, however, the scale is a single crescent-shaped structure situated at the base of, and forming a partial collar around about two-thirds of the circumference of, the gynophore (Text-fig. 22). Owing to two shallow clefts in its central region, the scale is provided with two lateral broader lobes and a narrower central lobe (Text-fig. 24). It measures 0·5 mm. at its greatest height, is located on the adaxial side of the gynophore, and gradually tapers away on approaching the abaxial side. The scale is markedly thickened at the base on the adaxial side, where an additional but much smaller cushion of tissue is located (Text-figs. 40 and 43). Microscopic examination of the adaxial and abaxial surfaces of this scale and cushion shows that the greater part of the area is composed of protruding secretory cells, either isolated or in groups of three or four, imparting to the whole surface a modified botryoidal appearance (Text-figs. 40, 41 and 43). These cells stain very deeply with safranin or Haidenhain's iron-alum haematoxylin stains. Clearly then this scale, with its adjoining cushion, is a nectar-secreting organ.
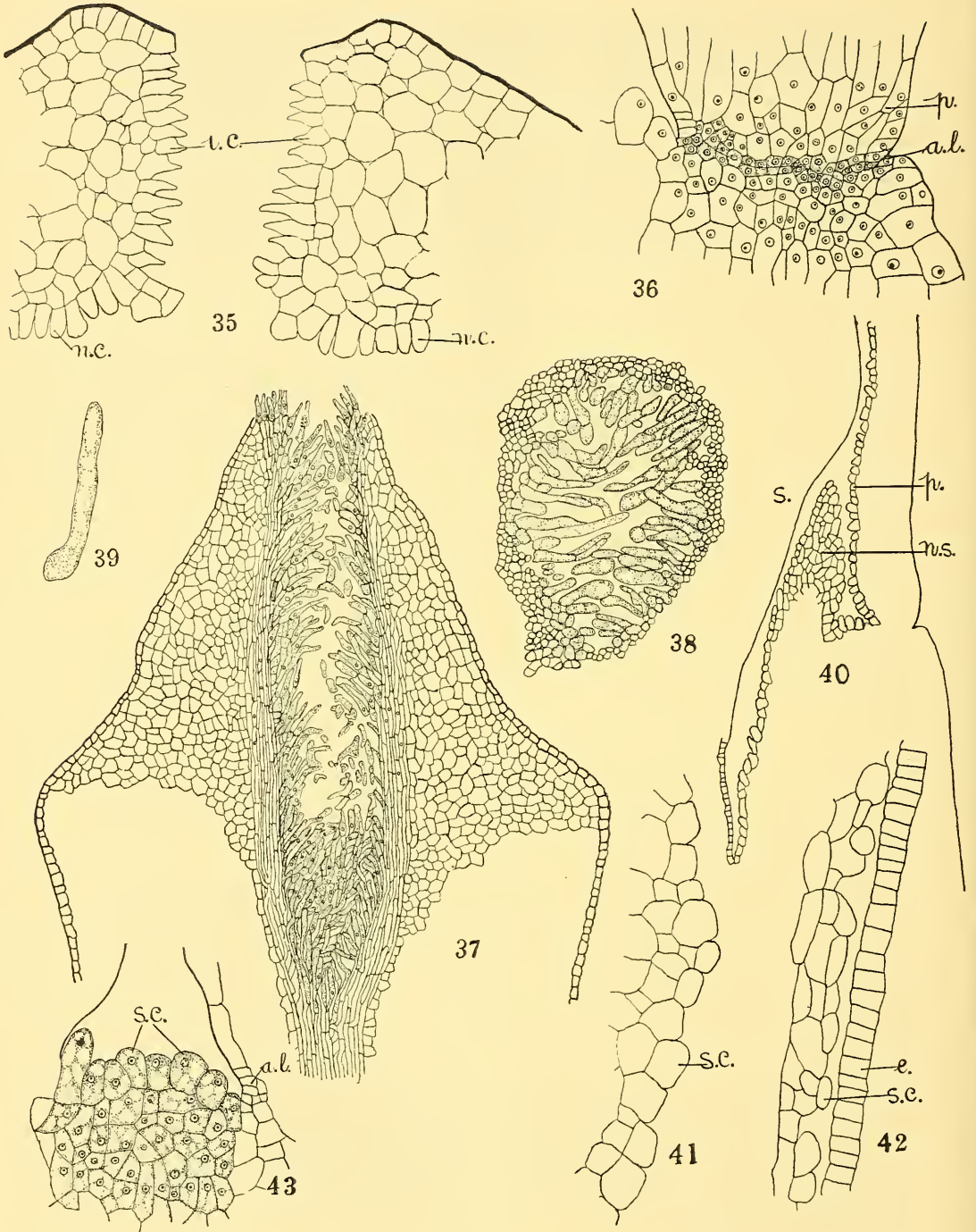
But secretion is not confined to this region, for an investigation of the basal part of the perianth segments in longitudinal section (Text-figs. 40 and 42) and in surface view shows that the majority of the epidermal cells protrude as finger-like processes, their length in many cases being several times their breadth.

Such cells do not take up a position at right angles to the perianth segment, but are applied for the most part to its surface in an irregular fashion. This perianth secretory region extends from the base of each segment to a height of 1·5 mm., so, taking into account the great volume of the protruding cells as well as the extensive area bearing them, it seems certain that the amount of nectar derived from this region must considerably exceed that which has its origin in the scale.

The combined areas of the secretory cells referred to are relatively very considerable, and their activity readily accounts for the exceptionally large production of nectar in the individual flower. Thus the nectar-scale is not—contrary to expectation—the sole or even the main source of nectar secretion, but rather plays a subsidiary rôle in this regard.

On the adaxial side of the flower a natural reservoir for holding the nectar is formed between the gynophore and the adhering bases of the opposing perianth segments, and also between the gynophore and the scale (Text-figs. 23 and 40).

Morphology of the Nectar-Scale.

The fact that the nectar-scales are, in this Family, often four in number, and alternating with the perianth segments, suggested to the writer that they might constitute a reduced and modified inner whorl of floral leaves, or in other words represent the vestiges of an ancestral normal corolla, in which case the present perianth would fall to be interpreted as the calyx.

In an endeavour to settle this question, which turns on the morphology of the nectar-scales, series of transverse (Text-figs. 22–24) and longitudinal sections (Text-figs. 33, 34, 40 and 43) of flower buds at successively older stages of development were examined. In every case the scale and cushion were found to

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Text-fig. 35.—Transverse section through portions of two adjoining perianth segments, showing interlocking marginal cells, *i.c.*, and nectar-secreting cells, *n.c.* × 225.

Text-fig. 36.—A longitudinal section showing cells of absciss layer, *a.l.*, at base of perianth segment, *p.* × 225.

Text-fig. 37.—Median longitudinal section of the cone-shaped apex of the style. The papillate nature of the stigmatic cells lining the central funnel-shaped cavity is depicted. Some of the receptive stigmatic cells are protruding beyond the rim of the cavity. × 135.

Text-fig. 38.—Transverse section of stigmatic cavity. × 225.

Text-fig. 39.—Typical form of a stigmatic cell; the vacuoles are apparent. × 550.

Text-fig. 40.—A longitudinal section through a nectar scale, *n.s.*, and base of a perianth segment, *p.* The protruding nature of the secretory cells covering the whole of the former, and lining the inner surface of the latter are indicated; *s*, style. × 38.

Text-fig. 41.—Longitudinal section of the marginal region of a nectar-scale showing secretory cells, *s.c.*, of epidermis. × 225.

Text-fig. 42.—Longitudinal section cut in the region of the ovary showing nectar-secreting epidermal cells, *s.c.*, of perianth adjoining epidermis, *e.*, of style. × 225.

Text-fig. 43.—Longitudinal section of cushion of tissue at base of nectar-scale showing general nature of the nectar-secreting cells, *s.c.*; a few of the cells of the absciss layer, *a.l.*, are evident. × 225.
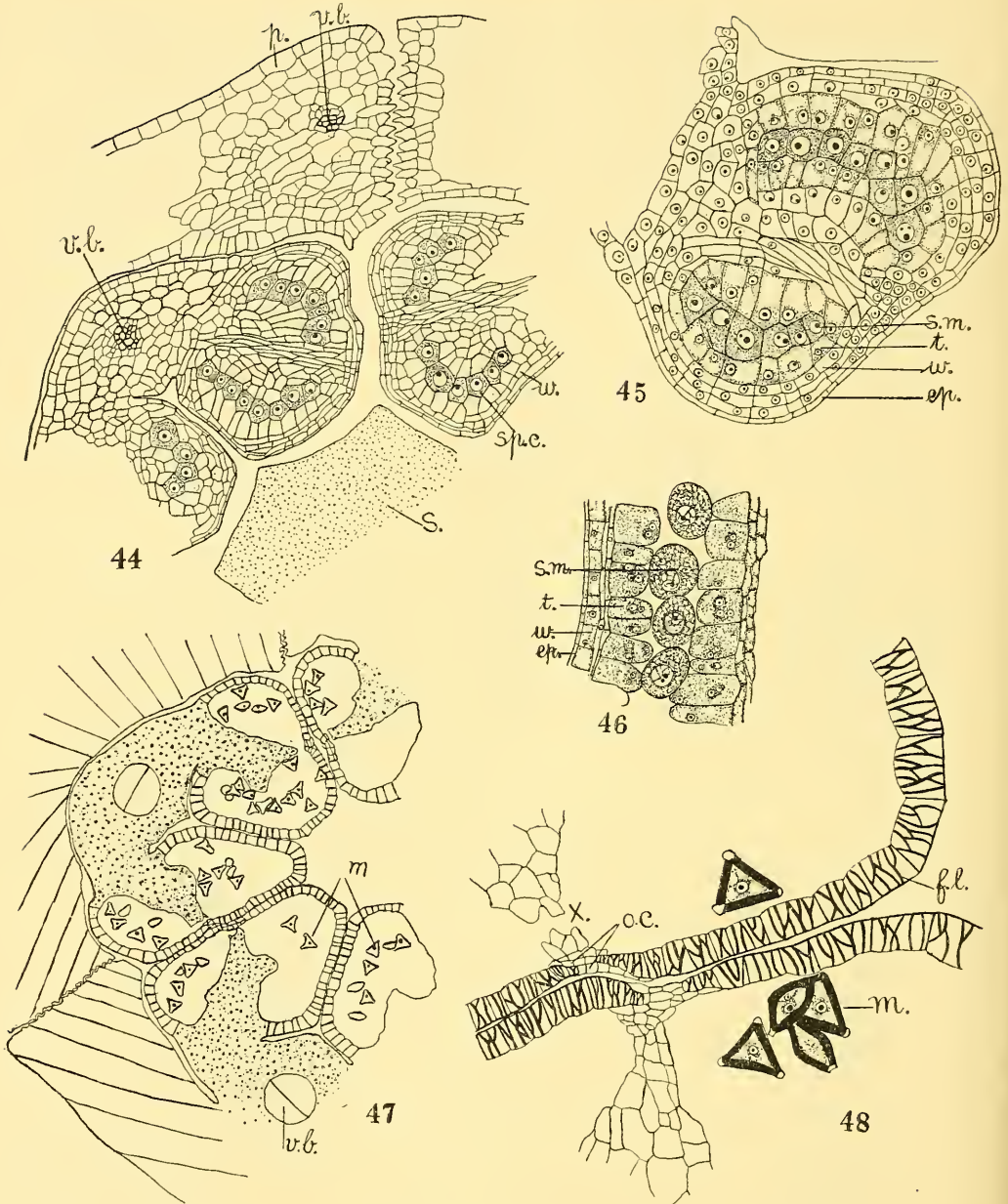
Text-fig. 44.—Transverse section of young flower bud showing portion of two perianth segments, *p.*, two adjacent anthers in part and the central style, *s.* *sp.c.*, sporogenous cells; *w.*, wall cells; *v.b.*, vascular bundle. × 135.

Text-fig. 45.—Transverse section of young anther slightly older than in preceding figure. *ep.*, epidermis; *t.*, tapetum; *w.*, intermediate wall layers; *s.m.*, microspore mother-cells. × 225.

consist entirely of thin-walled parenchymatous cells without any differentiation, except for the papillate nature of the epidermal secretory cells already discussed. There is no indication, whatsoever, of any associated vascular tissue, and consequently no ground for interpreting the scale as the morphological equivalent of a reduced perianth. It would seem then to be merely an adventitious accessory development of the torus.

The Microsporangium: Development and Structure.

The anther consists of four microsporangia held together by the connective tissue of the filament. Examination of sections of anthers at various stages showed that in the main the development conforms to what may be termed normal. Nevertheless, features of peculiar interest are encountered. For example, the archesporium becomes differentiated in the microsporangium of a flower bud about the stage represented in Text-figure 4. It is diagnosed as a plate of hypodermal cells, one cell thick, extending practically throughout the whole length of the anther. These cells are slightly radially elongated, possess large nuclei and have dense cytoplasm. Later, each of the archesporial cells divides by a periclinal wall, thereby giving rise to a primary parietal and a primary sporogenous layer respectively. The former, by active periclinal and anticlinal divisions, initiates a wall four cells thick lined internally by a fifth layer, the tapetum (Text-figs. 44, 45), easily recognized by its large, radially elongated, and densely staining cells. Meantime the primary sporogenous layer remains almost quiescent, the only notable change being an increase in the size of the constituent cells which, however, do not normally divide in order to produce an increased number of sporogenous cells, but, instead, function directly as spore mother-cells. It may be mentioned that occasionally an individual primary sporogenous cell does divide to give rise to two derived sporogenous cells, but such is a rare occurrence.

At first the spore mother-cells are closely packed and polygonal (Text-fig. 45), but, with the enlargement of the sporangial cavity consequent on a general increase in size of each microsporangium, they become free from one another, assume a rounded form, and float in the nutrient medium produced by the enlarged, actively secreting and now multinucleate cells of the tapetum (Text-fig. 46).

A typical microspore mother-cell is figured in Text-figure 49, where the characteristic large nucleus, coiled chromatin, nucleoli, dense cytoplasm and thin wall are evident.

Later the condition known as synizesis (Text-fig. 50) was diagnosed, and in slightly older material the various phases in reduction division were traced. The

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Text-fig. 46.—Longitudinal section of a microsporangium at a still older stage. *ep.*, epidermis; *t.*, tapetum; *w.*, wall layers; *s.m.*, free microspore mother-cells. × 225.

Text-fig. 47.—Tranverse section of two adjacent anthers at the stage just prior to dehiscence. The tissue separating adjoining microsporangia has been ruptured, and the region of the slit of dehiscence is evident. Portion of two perianth segments is shown; *m.*, tetrahedral microspores; *v.b.*, vascular bundle of connective. × 55.

Text-fig. 48.—Transverse section of part of each of two adjacent anthers. The rupture of the tissue separating two microsporangia of an anther is indicated at *x*. The epidermal cells have collapsed at this stage, and the fibrous layer, *f.l.*, now delimits the sporangium. The overlapping unthickened terminal cells, *o.c.*, of the fibrous layer, which later split apart and thus provide a longitudinal slit of dehiscence, are delineated. *m.*, tetrahedral microspores in binucleate condition. × 225.

G
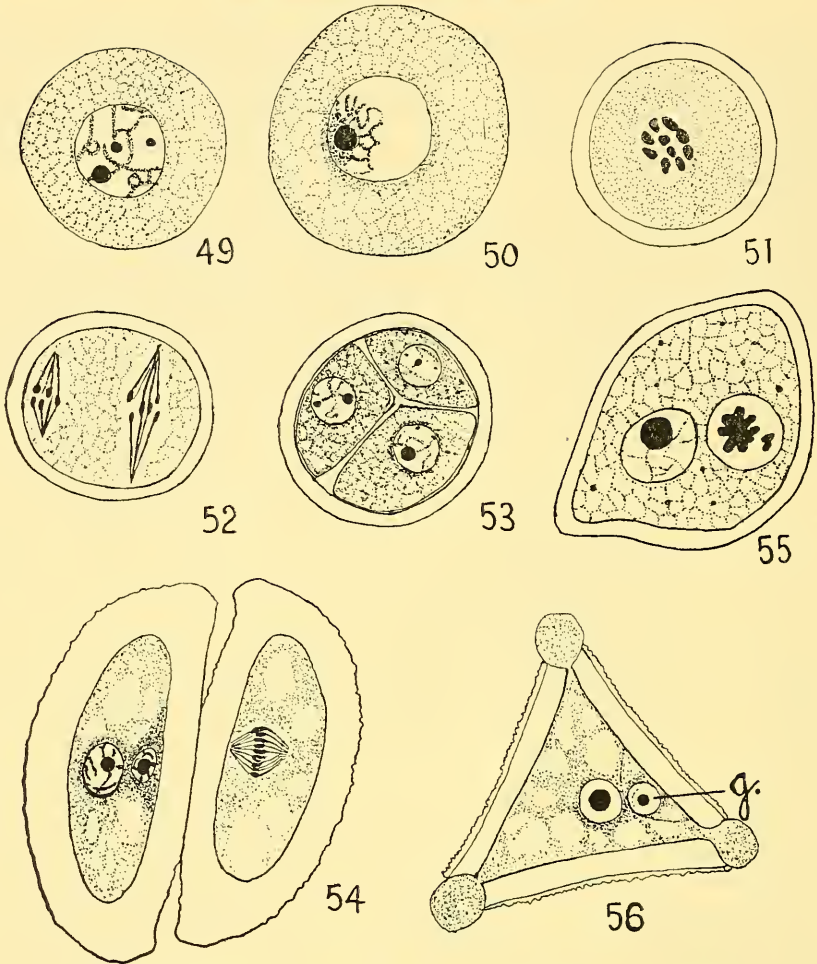
Text-fig. 49.—Microspore mother-cell as in Text-fig. 46. × 1,000.

Text-fig. 50.—Slightly older microspore mother-cell in stage known as synizesis. × 1,000.

Text-fig. 51.—Section of microspore mother-cell cut at right angles to the reduction division spindle in the late anaphase. The individual chromosomes, 10 in number, are shown. × 1,000.

Text-fig. 52.—Microspore mother-cell containing spindles of two nuclei dividing simultaneously. × 1,000.

Text-fig. 53.—Three microspores of a tetrad still contained within wall of mother-cell. × 1,000.

Text-fig. 54.—Two adjacent pollen grains *in situ* in a microsporangium. The walls have become much thickened. The spore on the left has reached the binucleate condition, while the nucleus of the other is in the metaphase of division. In section the shape of the spore depends on the plane in which it is cut. × 1,000.

Text-fig. 55.—A pollen grain at the binucleate stage. Only one nucleus containing a large nucleolus is present in this section. The other body, which simulates a nucleus, is a vacuole containing deeply staining bodies. × 1,000.

Text-fig. 56.—Mature binucleate microspore at dehiscence stage of pollen sac. A thick exine with outer layer of cutin, and the thin intine, protruding at the corners of the spore, are indicated. At this stage a thin membrane separates the smaller generative cell, *g.*, from the larger tube nucleus. × 1,000.

number of chromosomes in the haploid condition was determined as ten, this number being specially well demonstrated in sections cut at right angles to the spindle in the late anaphase (Text-fig. 51). At this period the wall of the spore has increased in thickness. The daughter nuclei divide immediately after formation, the spindles of this phase—one of which is oblique—being shown in Text-figure 52. Each of the resulting nuclei then becomes enveloped in a quarter-share of the cytoplasm, which in turn is bounded by a thin membrane, and thus the tetrad condition is attained (Text-fig. 53).

While microsporogenesis is proceeding, certain well-marked changes fall to be recorded in the development of the tapetum and wall of the sporangium. To begin with, the cells of the hypodermal layer become characterized by an increasing number of starch grains, which later furnish material for the pronounced fibrous thickenings characteristic of this layer at a more mature stage (Text-fig. 48). Meanwhile the tapetal cells enlarge (Text-fig. 46), become multinucleate, and gradually absorb the wall layers within the endothecium. However, after the inception of the microspores, the vitality of the tapetum steadily decreases, and by the time anther dehiscence is imminent, only a few fragmentary remains mark a hitherto prominent feature of the sporangium.

Another change, contemporaneous with the decline of the tapetum, is the collapse and gradual disappearance of the somewhat thin-walled and fragile cells of the original epidermis, so that, in the mature microsporangium (Text-figs. 47, 48), the fibrous layer delimits, and, for all practical purposes, constitutes the free portion or arms of the sporangial wall.

A similar occurrence has been recorded in *Dampiera stricta* (Brough, 1927, p. 480), while in *Styphelia longifolia* (Brough, 1924, pp. 166 and 167) the fibrous layer is morphologically the epidermis. This progressive relegation of the endothecium to the outside of the mature sporangium is no doubt related to the hot, dry conditions commonly prevailing at the time of anthesis in many Australian flowers. Reference to Text-figure 47 shows the general structure of the anther just prior to anthesis.

The partition wall separating the pairs of microsporangia has ruptured, resulting in the formation of two pollen sacs in each anther. An examination of the fibrous layer indicates a somewhat noteworthy and peculiar dehiscence mechanism. It is observed that at *o.c.* in Text-figure 48, where the endothecial layer of one microsporangium meets the corresponding layer of the adjoining sporangium, a double layer of cells devoid of fibres is formed by the overlapping of the tapering ends of the respective endothecial layers. As usual, then, a region of weakness extends throughout the length of the pollen sac, but at dehiscence, instead of the rough fracture, so commonly found in anthers, there is an orderly separation of the cells along the line of the overlap, and a subsequent pulling apart of the two freed arms, each terminated by a row of unbroken tapering cells—a somewhat unique phenomenon in anther dehiscence (Text-fig. 32).

The Male Gametophyte.

After the uninucleate microspores have been set free by the dissolution of the wall of the mother-cell, they rapidly increase in size and gradually assume a precise tetrahedral form in the nearly mature flower bud (Text-figs. 48, 56). This shape is characteristic of most Protead forms. Soon after this, but before anthesis, the nucleus undergoes mitotic division, and the binucleate stage is attained, the two nuclei being separated by a thin membranous wall (Text-figs. 54, 56). The

generative nucleus is the smaller and is more densely encased in cytoplasm than the larger centrally placed vegetative nucleus.

Meanwhile, the wall becomes excessively thickened, the massive exine being differentiated into two layers, a clear outer covering of cutin and a thicker and deeply staining inner layer. A thin membrane represents the intine (Text-fig. 56). This thickening of the exine is not uniform, however, and is distributed in such a manner that four unthickened areas are evident at the points of the tetrahedron.

Just prior to anthesis one or more vacuoles make their appearance in the cytoplasm of the pollen grain. In preparations stained by Haidenhain's iron-alum haematoxylin method it was observed that such vacuoles contain numerous deeply-stained granules (Text-fig. 55) which may be interpreted as reserve food material—a useful adjunct in the nutrition of the relatively long pollen tube. As such vacuoles are of large size relative to the microspore as a whole, the consequent increase in internal pressure is no doubt the cause of the protrusion of the intine at the four corners of the tetrahedron (Text-fig. 56). Such vacuolation seems to be a constant prelude to spore germination, for it is at this stage of development that the style frees itself from the perianth, and raises the pollen aloft on its distal extremity.

On germination, one of the protuberances grows out to form a pollen tube, and its subsequent development agrees closely with that previously described in the case of *Dampiera stricta* (Brough, 1927, p. 482). It may be added that the thickening of the spore wall is more pronounced than in *Dampiera*.

Examination of the receptive stigma in longitudinal section revealed pollen grains in situ. These germinate, as indicated, and subsequently pollen tubes traverse the long style and eventually were observed to enter the ovarian cavity, at the chalazal region of the two pendulous ovules (Text-fig. 34). The pollen tube pursues its course downwards, adhering to the thin-walled slightly papillate cells lining the ovary wall, and evidently deriving nourishment therefrom. The space between the wall and the ovules is just sufficient to permit easy passage of the pollen tube which, on reaching the lower end of the ovarian cavity, turns almost at right angles and thereby comes into contact with the apical region of the pendulous ovule, along which it grows until the neighbourhood of the micropyle is reached. The micropyle is a relatively wide funnel-shaped passage, easy of access. The cells of the inner integument bordering the upper region of the micropyle are of a strikingly papillate nature and admirably adapted to stimulate further growth of the pollen tube, which eventually penetrates the crushed cells of the nucellus, and enters the embryo sac in the normal way.

The Ovule.

The gynoecium is represented by a single carpel occupying the organic apex of the thalamus (Text-figs. 5–9). Transverse and longitudinal sections both demonstrate the single foliar nature of this organ which is therefore mono-carpellary.

The vascular supply in the very young carpel is at first represented by a single median vascular strand, but as development proceeds lateral veins arise, which in turn branch, and eventually send vascular extensions to the carpellary leaf margin (Text-figs. 12–14).

At a very young stage a slight protuberance is noticeable near the base of each of the infolded margins of the carpel. This is due to the meristematic

activity of the densely cytoplasmic cells of this region. Evidently such expansions represent the combined young placentae and incipient nucelli, the latter of which protrude slightly into the ovarian cavity (Text-fig. 12, at x). There is no very decided placenta, a fact which is attested by the study of similar sections through older material (Text-figs. 13, 16 and 33). In Text-figure 13 the impinging but distinct epidermal layers of the inrolled margins of the carpel are clearly demonstrated. Gradually the nucellus and short funiculus become differentiated (Text-figs. 13, 15 and 33) and a slight curvature (Text-fig. 16), indicative of the final anatropous condition, becomes manifest. The integuments, however, are still undeveloped. As growth proceeds the curvature of the ovule becomes more apparent, and is steadily accentuated, until the relatively mature phase demonstrated in Text-figures 57 and 58 is arrived at.
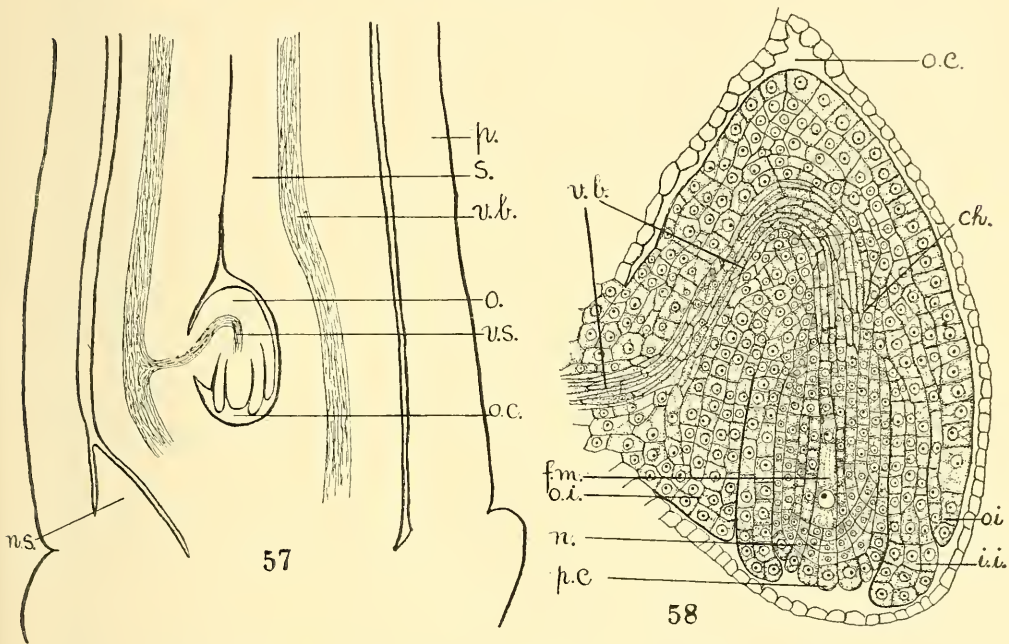
Text-fig. 57.—Median longitudinal section cut in antero-posterior plane of the lower part of a flower bud showing ovule in ovarian cavity. p., perianth; s., style; v.b., vascular strand of carpel; v.s., vascular strand from carpel passing to chalaza of ovule, o.; o.c., ovarian cavity; n.s., nectar-scale. × 55.

Text-fig. 58.—Median longitudinal section of an ovule showing general structure and functional megaspore which later develops into the female gametophyte. o.c., ovarian cavity; ch., chalaza; v.b., vascular bundle; o.i., outer integument; i.i., inner integument; f.m., functional megaspore; n., nucellus; p.c., papillate cells of nucellus. × 225.

Herein is evident the essential gross structure of the young ovule—the short, thick funiculus with central vascular bundle, the massive nucellus, and two partially developed integuments.

Now, the mature flower is bilaterally symmetrical, and the margins of the carpel fuse on the adaxial side. The two ovules of each ovary are right and left of the median plane of symmetry which is antero-posterior. Accordingly, in the

case of longitudinal sections the cut must be made at right angles to this plane, if both ovules are to be exposed in a single section (Text-figs. 11 and 34). However, in addition to this, there is the fact that the somewhat flattened ovules are orientated to lie in the antero-posterior plane, so that this plane must be followed in section when longitudinal median sections of the ovule are required. Such a section will of course only pass through one ovule at a time (Text-figs. 33, 57, 58).

A noteworthy feature of this particular ovule is the relatively late inception and slow development of the integuments. For example, at the stage when the megaspore mother-cell has become differentiated (Text-fig. 16), there is merely a slight indication at x of the region of origin of the inner integument. Even at a much older stage (Text-fig. 58) where the functional megaspore has become apparent, the integuments are far short of completely enclosing the nucellus, and it is not until the eight-nucleate stage of the female gametophyte has been realized, that the nucellus becomes completely invested. At the stage of development indicated in Text-figure 58, the outer integument is two, and the inner integument three cells thick, but as maturity of the female gametophyte is attained, the inner integument typically increases to five cells broad towards its apex, while the outer integument increases to eight cells in thickness (Text-fig. 71). So far then, the integuments are comparatively thin structures.

The nucellus is of the massive type, and the manner of its development by the very regular formation of periclinal and anticlinal walls is indicated by the uniform arrangement of its constituent cells (Text-figs. 16 and 58). The exposed epidermal cells of the nucellus are distinctly glandular in appearance (Text-fig. 58), and remain so until after fertilization. This structural feature is, as already mentioned, also characteristic of those cells of the inner integument which line the micropyle, especially those bordering the rim.

It is difficult to dogmatize on the particular rôle of such cells, but it may be that they secrete a liquid whose chemotactic influence guides the pollen tube direct to the micropyle. In any case they are eminently suited to facilitating the progress of the pollen tube towards the embryo sac.

Ballantine (1909, p. 161) calls attention to the occurrence of such cells in *Protea Lepidocarpon*, and their presence in the ovules of different genera, and from plants peculiar to two such widely separated continents as Africa and Australia, is both interesting and suggestive.

Subsequently, the ovule increases greatly in size, the increment being primarily due to the extraordinary activity of a zone of meristematic cells situated in the chalazal region of the ovule. The ovule steadily increases in all dimensions, but the most noteworthy feature of this later development is the marked increase in length of the ovule, which now assumes an elongated, flattened form.

Megasporogenesis.

Ballantine (1909) has already furnished, inter alia, an account of the development of the megaspore mother-cell, spore tetrad, functional megaspore and embryo sac of a Protead form, *Protea Lepidocarpon*, and in consequence the present writer will only present for purposes of comparison the more significant features of these particular phases of the life-history of *Grevillea robusta*.

At the stage in development of the nucellus indicated in Text-figures 16 and 26 respectively, the megaspore mother-cell is recognized by its relatively greater size and large nucleus. It is also depicted in transverse section in Text-figures 59 and 60. Although only one such cell is fully differentiated, it is to be noted that some

of the contiguous cells are slightly larger and more densely cytoplasmic than the remainder of the cells of the nucellus. The nature of these cells surrounding the mother-cell suggested that there might be a plurality of mother-cells in *Grevillea robusta*, but careful search failed to reveal any evidence of such. Ballantine (1909, p. 161) refers to this feature when he observes, "a small group of large cells situated below the hypodermal layer includes one which becomes the megaspore mother-cell". The mother-cell is deeply sunken, and occurs in what may be termed the distal region of the sporogenous tissue, as opposed to the purely sterile region of the regular wall layers.

The development of the megaspore mother-cell results in the formation of four megaspores (Text-figs. 61, 62) which at this stage are arranged in descending order of magnitude, the largest adjoining the chalazal region of the ovule. The spores are separated by thin walls. The innermost spore steadily increases in dimensions, and eventually supplants those more superficially placed (Text-fig. 63).

The germination of the functional megaspore (Text-figs. 58, 64) is heralded by a marked increase in volume, while vacuoles become increasingly apparent in the cytoplasm. The nucleus divides to give the binucleate stage of the female gametophyte (Text-fig. 65), and thereafter development proceeds along normal lines until the eight-nucleate stage is attained (Text-fig. 66). Meanwhile the sac has increased in all dimensions, and continues to do so, but more particularly in length, until at maturity (Text-figs. 70, 71) it assumes a much elongated cylindrical form, a shape which is in conformity with the elongating nucellus.

The Female Gametophyte.

The mature gametophyte is depicted in Text-figure 66. The egg-apparatus consists of three cells arranged in typical fashion. Each of the two synergids is pyriform in shape, has a large central vacuole, and contains a relatively small nucleus. The ovum about the time of fertilization is encased in an aggregation of starch grains (Text-fig. 68). The polar nuclei first come into contact slightly to the micropylar side of the middle of the embryo sac, but later change their position, actual fusion—various stages of which were observed—occurring in the immediate neighbourhood of the ovum (Text-fig. 69). The antipodal cells are three in number, separated by walls and located as usual in the lower and somewhat narrowed end of the sac (Text-figs. 66, 67). They are still evident during the earlier stages of endosperm formation, but do not enlarge, and clearly are of little significance. Megasporogenesis, then, and the structure of the mature gametophyte conform to what may be designated normal, and agree very well with what has been described in the case of *Protea Lepidocarpon*.

Fertilization.

The pollen tube, as already described, has been seen to enter the micropyle, where it comes into contact with the somewhat glandular cells lining the rim and cavity of the passage. Such cells no doubt contribute to the nourishment of the tube, which eventually passes through the thin tissue of crushed cells of the nucellus capping the female gametophyte, and eventually enters the embryo sac. The discharge of the male gametes into the sac was not actually seen, but in one preparation a crushed synergid, and two small bodies, one in the neighbourhood of the egg, and another in close proximity to the two polar nuclei (Text-fig. 69), at least suggested normal fertilization of the egg and the occurrence of triple fusion. These phenomena were not, however, diagnosed with certainty.
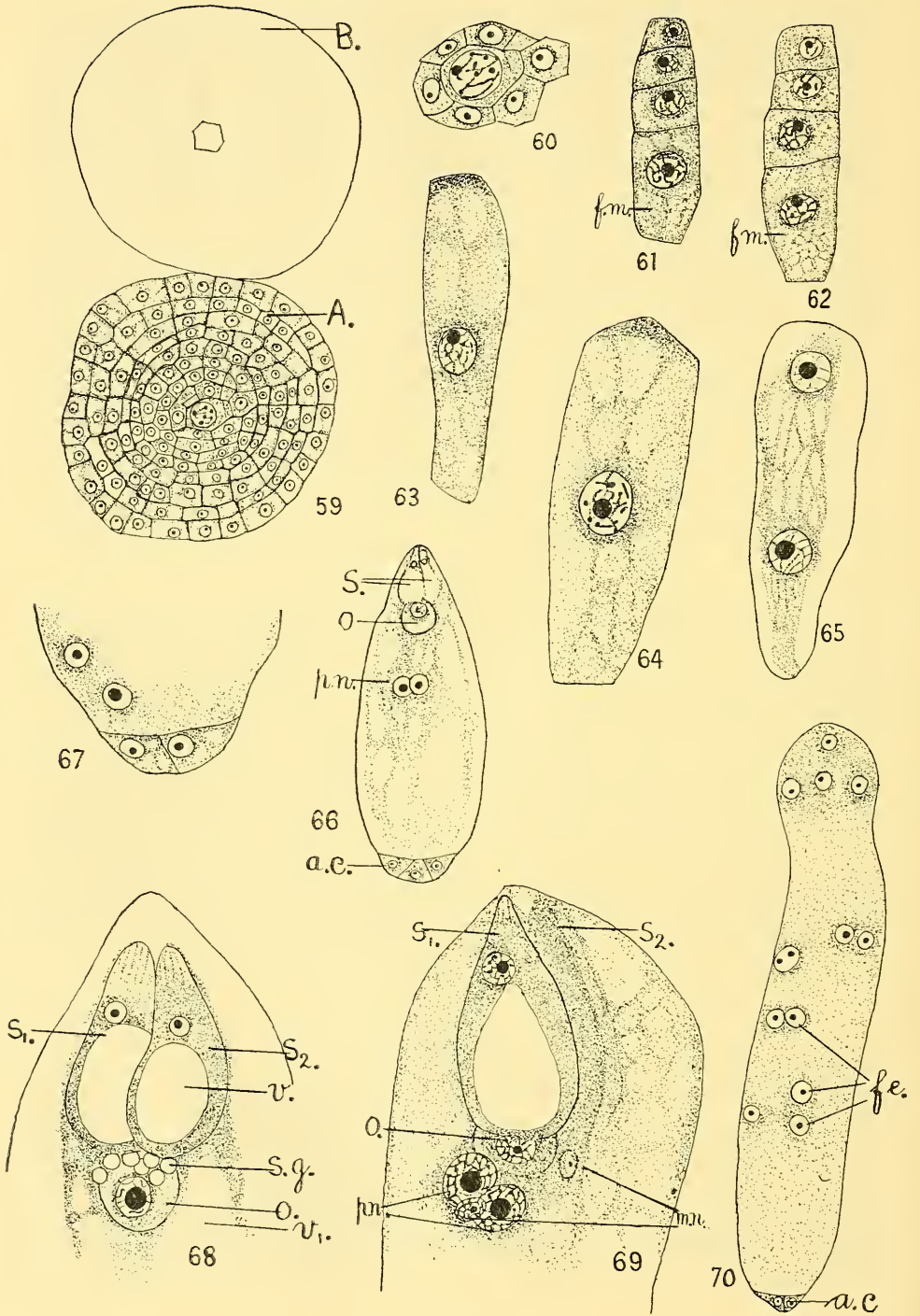
Endosperm.

The endosperm nucleus divides before that of the oospore, and subsequent mitotic activity results in the formation of a large number of nuclei in the sac while the egg is still quiescent, or merely increasing in size. Such early endosperm nuclei (Text-fig. 70) are, however, practically confined to the upper two-thirds of the sac in which region wall formation commences, and forms a tissue around the zygote (Text-fig. 74). Meanwhile, the antipodal cells are unchanged. Subsequently, they neither divide nor increase in size, and so may now be dismissed, as of no further significance. Thereafter endosperm formation proceeds slowly in the direction of the chalaza, but is now closely pursued by the rapidly developing embryo. The sluggish development of the endosperm in the chalazal region of the sac is a marked feature, and even when mature this tissue often fails to fill completely this lower end of the sac, which is often seen to be devoid of tissue, and to collapse, when longitudinal sections of almost mature seeds are under examination. The consequence of this behaviour, coupled with the disintegration of the nucellus, is that the mature embryo is somewhat loosely encased by the testa of the seed, which, owing to the complete absorption of the endosperm during the final development of the embryo, becomes non-endospermic at maturity.

The Embryo.

Soon after fertilization it is observed that the nucleus of the egg is considerably greater in magnitude than that of the ovum from which it was derived (Text-fig. 72). In addition, the oospore modifies its position, and moves in the direction of the micropyle, for it is next seen in contact with the micropylar end of the sac (Text-fig. 74). The cause of this change in position is somewhat obscure, but one may hazard the opinion that it may be partly a natural response to the disintegration of the synergids, and partly due to increased pressure consequent on the active division of the endosperm nucleus on the side of the

Text-fig. 59.—Transverse section through two impinging ovules, A and B, of an ovary. In the centre of A is seen the large megaspore mother-cell enclosing which are several cells more densely cytoplasmic than those more remote. The regular radial arrangement of the cells of the nucellus is very evident. × 300.

Text-fig. 60.—Megaspore mother-cell more highly magnified than in previous drawing. × 730.

Text-fig. 61.—A megaspore tetrad. *f.m.*, the functional megaspore adjoining the chalaza. × 730.

Text-fig. 62.—As above, but at an older stage; all the megaspores have enlarged. *f.m.*, functional megaspore. × 730.

Text-fig. 63.—The functional megaspore; the other three megaspores have been crushed and absorbed. × 730.

Text-fig. 64.—The young uni-nucleate embryo sac. Vacuolation has commenced. × 730.

Text-fig. 65.—Binucleate stage of embryo sac. × 730.

Text-fig. 66.—Mature embryo sac. *s.*, synergids; *o.*, ovum; *p.n.*, polar nuclei in contact; *a.c.*, antipodal cells. × 300.

Text-fig. 67.—Lower part of embryo sac showing two of the antipodal cells, and free endosperm nuclei. × 430.

Text-fig. 68.—Micropylar end of embryo sac with egg apparatus. s_1. and s_2., synergids; *o.*, ovum; *s.g.*, starch grains; *v.*, vacuole of synergid; v_1., vacuole of embryo sac. × 730.

Text-fig. 69.—Micropylar end of embryo sac showing one normal synergid, S_1., one disintegrated synergid, S_2., and ovum, O., two polar nuclei, *p.n.*. and two problematical male nuclei, *m.n.* × 730.

Text-fig. 70.—Longitudinal section of embryo sac after fertilization with free endosperm nuclei, *f.e.*, and two antipodal cells, *a.c.* × 180.
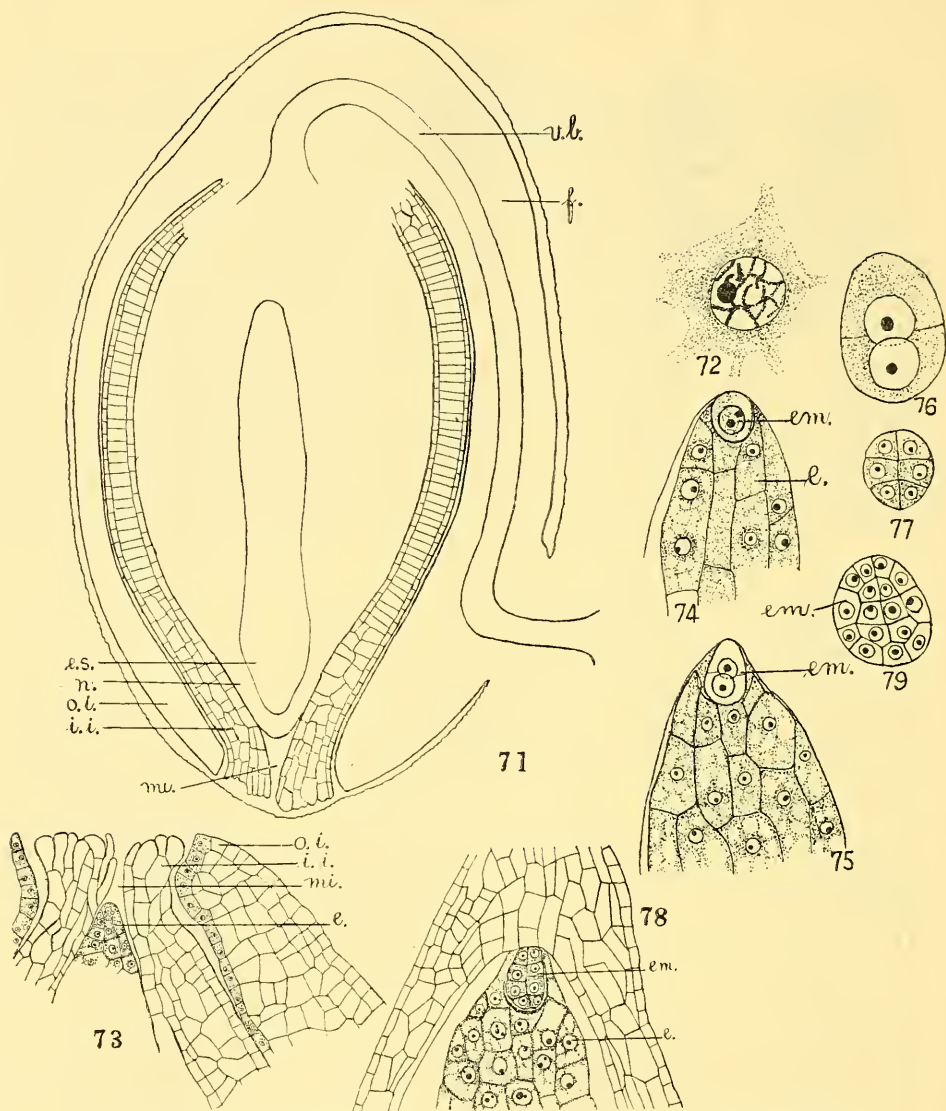
Text-fig. 71.—Longitudinal section cut in antero-posterior plane of seed not long after fertilization. *o.i.,* outer integument; *i.i.,* inner integument; *mi.,* micropyle; *n.,* nucellus; *e.s.,* embryo sac; *v.b.,* vascular bundle of funiculus, *f.* × 120.

Text-fig. 72.—Nucleus of egg just before latter moves to tip of embryo sac adjoining micropyle. × 730.

Text-fig. 73.—Longitudinal section through micropylar end of seed. The endosperm protrudes slightly into the micropyle. *o.i.,* outer integument; *i.i.,* inner integument; *mi.,* micropyle; *e.,* endosperm. × 120.

Text-fig. 74.—Longitudinal section through micropylar end of sac showing zygote, *em.,* amid endosperm tissue, *e.* × 300.

Text-fig. 75.—Two-celled stage of embryo, *em.,* surrounded by endosperm. × 300.

Text-fig. 76.—Two-celled embryo, as in previous text-fig., showing wall separating the nuclei. × 730.

Text-figs. 77-82.—Series of figures illustrating successive stages in embryogeny. For explanation see text. *em.,* embryo; *e.,* endosperm; *w.,* wing; *o.i.,* outer integument; *i.i.,* inner integument. Figs. 77, 79 × 300; fig. 78 × 200; figs. 80-82 × 225.
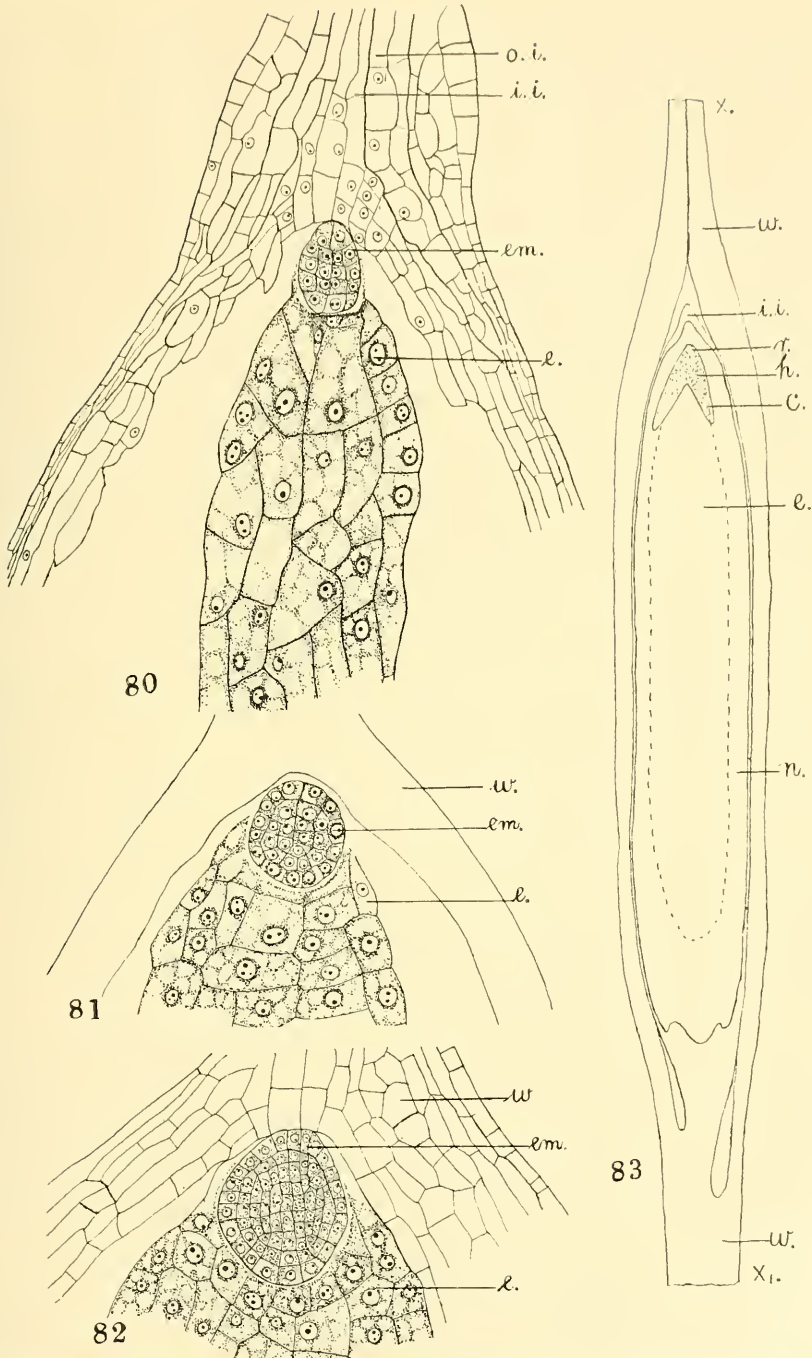
Text-fig. 83.—Longitudinal section of immature seed cut in lateral plane. The embryo has differentiated into two cotyledons, *c.*, hypocotyl, *h.*, and radicle, *r.* The wing is only shown in part, being incomplete at X and X₁. *w.*, wing; *i.i.*, inner integument; *e.*, endosperm; *n.*, remains of nucellus. × about 15. This drawing is not camera lucida.

egg remote from the micropyle. Certainly endosperm formation anticipates the division of the zygote, a fact which becomes apparent on consulting Text-figure 74, where the undivided oospore is seen embedded in endosperm tissue.

After division of the nucleus of the oospore, a wall is formed at right angles to the long axis of the ovule. This gives the bicellular stage indicated in Text-figures 75 and 76. Ensuing divisions lead to the formation of a flat and approximately oval plate of cells (Text-figs. 78, 80), steadily invading the endosperm tissue, which is being brought into solution by the secretion of an enzyme, and subsequently absorbed by the growing embryo. The action of such an enzyme is amply demonstrated by the clear region fringing the embryo, and the corroded nature of the adjacent endosperm cells (Text-figs. 78, 80, 81, 82).

At the stage depicted in Text-figure 80 periclinal walls have resulted in the separation of a dermatogen, and inspection of successively older stages shows the differentiation of the plerome and periblem regions, accompanied by a change from the early oval form to one which is practically circular (Text-figs. 81, 82).

A noteworthy feature of this disc-shaped embryo is the entire absence of a suspensor. The embryo at its inception takes up a position—at the micropylar end of the sac—which it never vacates. Such a disposition no doubt entails certain disadvantages from the point of view of easy nutrition, but this handicap evidently presents no real difficulty, since further development proceeds apace, and eventually results in the total destruction of the endosperm. During embryogeny the embryo sac and endosperm steadily increase in volume, and some slight invasion of the micropyle becomes manifest (Text-fig. 73).

The next significant phase is the differentiation of the main body regions—the two cotyledons, the plumule, hypocotyl and radicle (Text-figs. 83, 84). The last mentioned text-figure shows the adaxial surfaces of the two cotyledons, one of which has been detached from the young plant. A noteworthy and distinctive feature is the possession of two lobes at the base of each of the two cotyledons which enclose the plumule and hypocotyl. A clearer perception of the nature of this lobing—together with the general structure of the embryo—may perhaps be gained by examination of a mature seed in transverse section. Text-figures 86–88 show three such selected sections arranged in basipetal succession. In Text-figure 86 the two cotyledons with their vascular bundles are apparent. The succeeding drawing depicts the same cotyledons in the region where they are fused with the cotyledonary zone of the young stem, which is provided with a vascular system of four bundles.

The third section illustrates clearly the four lobes of the cotyledons. It will be observed that they are quite detached from the hypocotyl, and that the two lobes on the right belong to one cotyledon, while those to the left are connected with the other. Further, seeing that no leaves have yet grown from the plumule, and since the stele of the young stem shows a ring of four bundles, it follows that each cotyledon must send two separate vascular strands into the hypocotyl. Each of these bundles branches freely at the base of the broad, flat, oval cotyledon, as is illustrated in Text-figure 84, and so provides the vascular supply of the seed leaves.

The Seed: Development and Structure.

Just after fertilization the seed is long, and much flattened in the antero-posterior plane (Text-fig. 71). Consequently, in longitudinal section, its appearance varies according to the plane in which the cut is made. The nucellus is of the massive type. The inner integument is closely applied to the nucellus,

and in turn is encased in the outer integument, but it is only at the base of the ovule that these three components of the young seed are in organic connection: they are easily separated from each other by a little pressure.

The embryo sac extends from the micropylar region of the ovule to within a short distance of the chalaza, and in the young seed, when the embryo consists of only a few cells, is completely enveloped in nucellar tissue, which, however, thins out to a few layers at the upper end of the sac. Elsewhere the envelope of nucellar tissue is relatively broad in the antero-posterior plane, especially at and towards the chalazal end, where it forms a thick pad. Towards the periphery of this tissue its cellular nature at this stage is still fully preserved, but internal to this the cells have a corroded appearance which is gradually accentuated, until, in the immediate vicinity of the sac, a cellular structure is no longer apparent. It is surmised, therefore, that a powerful enzyme, capable of bringing the nucellar tissue into a soluble condition, is secreted by the sac, which consequently becomes suspended in a liquid nutrient medium.

As development proceeds and the endosperm and embryo become differentiated, fresh invasions are made on the nucellus, with the result that eventually the whole of the nucellar tissue—with the exception of the occasional occurrence of a few remnant cells adjoining the integument and chalaza—is destroyed.

The disintegration of the nucellus is accompanied by a tremendous increase in the size of the sac, but its increase in volume fails to keep pace with the space vacated by the nucellus, and consequently an unoccupied region is left between the sac and the integumentary tissue. This space is permanent, and persists even in the mature seed. A very striking feature, consequent on fertilization, is the very rapid and great increase in the dimensions of the seed (Text-fig. 83). This is due, for the most part, to the tremendous activity of a group of meristematic cells located in the basal region of the nucellus. This meristem is apparent even in the youngest stages of the ovule, and maintains its identity throughout. After fertilization the increase is chiefly in a direction parallel to the long axis of the seed, which consequently is of a much elongated and pendulous nature at maturity.

The Wing: Development and Morphology.

The mature seed is characterized by the possession of a broad, membranous wing (Text-figs. I (27) and 71, 73, 83 and 85–88) expanded in the same plane as the flattened body of the seed, and entirely encircling it. This structure contributes to, and emphasizes the outstandingly platyspermic nature of the seed.

The morphology of this structure demands elucidation. Is it integumentary or arilloid? In order to settle this question the development of the ovule was studied from its inception until it passed to the mature seed condition. Such an investigation showed that no enveloping structure, other than the integuments, ever arises. Accordingly, the wing is a specialized integumentary development. In the ovule the inner integument, as well as the outer, is considerably extended in the antero-posterior plane (Text-fig. 71), and this helps to emphasize the flattened nature of the ovule. This inner integument reaches its maximum soon after fertilization of the ovum is effected, and is then many cells in thickness in its broadest dimensions. Thereafter, post-fertilization growth of the tissues within, and resistance offered by the enveloping integument without, result in lateral crushing which is accentuated until, in the mature seed, only a thin, almost structureless tissue represents a formerly outstanding component of the ovule.
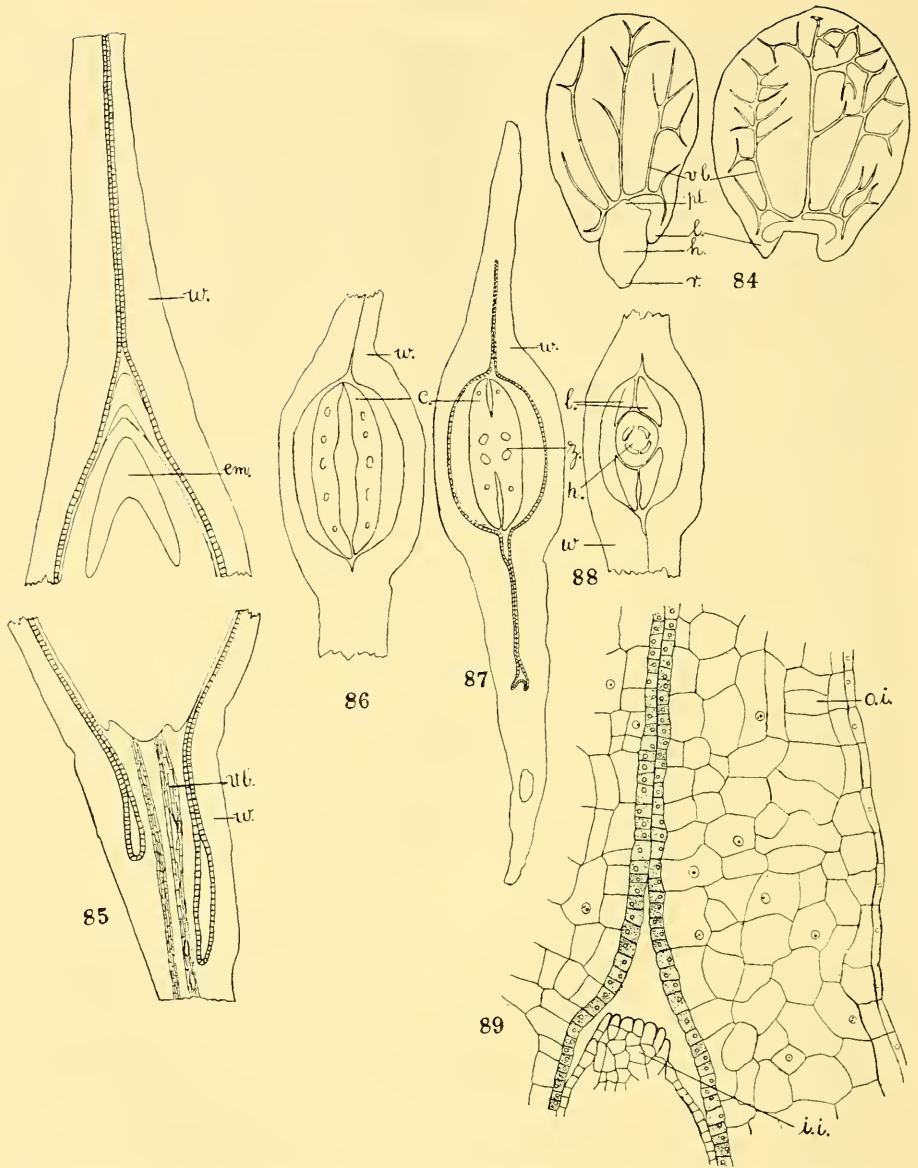
Text-fig. 84.—Embryo removed from seed. The cotyledon on the right has been detached from the young plant. The adaxial surfaces of the cotyledons are exposed. *pl.*, plumule; *h.*, hypocotyl; *r.*, radicle; *v.b.*, vascular bundles; *l.*, lobe of cotyledon. × 30.

Text-fig. 85.—As in Text-fig. 83, but on a higher scale of magnification and with the central portion of the seed omitted. *em.*, embryo; *w.*, wing; *v.b.*, vascular bundle. × 30.

Text-figs. 86-88.—Selected sections from a series of transverse sections of mature seed. For explanation see text. Only in Text-fig. 87 is the full extent of the wing shown. *w.*, wing; *l.*, lobes of cotyledon; *z.*, cotyledonary zone; *h.*, hypocotyl. × 30.

Text-fig. 89.—Longitudinal section, cut in lateral plane, through micropylar end of seed showing detailed structure of part of wing. *o.i.*, outer integument; *i.i.*, inner integument. × 110.

In the case of the outer integument, however, a very different fate falls to be recorded.

At the stage of ovule development illustrated in the text-figure last mentioned, its structure is very similar to that of the inner integument, but soon after fertilization, and at the stage when the endosperm has become conspicuous (Text-fig. 73), meristematic activity is noticeable in the row of cells next to those forming the inner limit of the integument. These divide rapidly and in orderly fashion, and thus form a much extended tissue. The radial arrangement of the cells resulting from this regular tangential division becomes more noticeable as development proceeds (Text-fig. 89). This lateral expansion of the integument continues, however, to be much more active in the antero-posterior plane, with the result that the integument assumes the flattened form characteristic of the wing in the mature seed.

The lateral involutions seen in the transverse section of the seed (Text-fig. 87) are due to the outer integument accommodating itself during later development to the original lateral expansion of the inner integument. Such expansion of both integuments in the antero-posterior plane is, as already stated, a feature of the ovule just prior to fertilization, but whereas the outer integument continues to increase in size, the inner integument suffers compression and practical obliteration, but none the less records its previous existence in the lateral involutions evident on each side of the mature seed when viewed in transverse section.

It must be emphasized that a very marked feature of the development of the seed as a whole is a great increase in length accompanied by excessive development in the antero-posterior plane. This latter feature is an outstanding characteristic of the development of each component part—nucellus, endosperm, embryo, integuments—of the ovule and seed, and accounts for its mature form. Accordingly, the pronounced flattened shape of this organ and the morphological nature of the wing have now been interpreted.

Pollination.

The anthers dehisce just prior to or simultaneously with the process of anthesis, so that when the style frees itself from the perianth segments, the pollen is poised as a cone-shaped mass on the distal end (Text-fig. I, 10). This occurs before the stigma is exposed. Accordingly pollen is prevented from coming into contact with the stigma of the flower which produces it. In other words, the flower is strictly protandrous. Practically all of this pollen is dislodged during the first day or two of its exposure, either by the numerous bird visitors, or by air currents. In any case, in the event of any pollen retaining its original position, it is certain to be dislodged—outwards and downwards—when at a later stage the growing stigmatic cells finally burst through the epidermal layer (Text-fig. 34) and first expose themselves to outside contact. The whole process is very much akin to the procedure during the pollination period of *Dampiera stricta* (Brough, 1927, p. 493).

On further development a wider crater-like cavity, which is lined by the long papillate cells of the stigma, is formed (Text-figs. 37 and 38). Some of these receptive cells protrude from the opening, and are in a suitable position for the reception of pollen. Owing to its general configuration and the nature of the cells composing it, such a stigma is eminently suited to the germination, nourishment and development of the young male gametophyte.

Below the stigma proper is located a tract of thin-walled elongated cells (Text-figs. 33 and 37) which traverses the length of the style, and provides an admirable conducting and feeding tissue for the passage of the pollen tube.

In the section of this research dealing with organogeny and general floral development, attention has already been drawn to certain abnormal flowers in which the perianth is not shed at the usual time, but persists as a cap enveloping the distal end of the style (Text-fig. I, A and B). One naturally wonders concerning the fate of such "capped" flowers, and the question arises is self-pollination effected, and if so, do embryos arise?

It so happened that the answer to such queries was suggested during the examination of another species of Grevillea, namely, G. Banksii, a species in which "capped" flowers (Text-fig. I, a–f) were found to be much more abundant than in the Silky Oak. Incidentally, it may be mentioned that observation showed that in G. Banksii seeds are set more abundantly than in any other species known to the writer. Here, in one selected case, 25 "capped" flowers, out of a total of 42 on a single inflorescence, were found to have full-sized fertile seeds, although the perianth still protected the stigma. This showed quite conclusively that self-pollination and fertilization were by no means uncommon in this species. In fact, from observations made it is computed that during the latter part of the seed-setting season thirty to forty per cent. of the seeds produced in this district by G. Banksii are derived from "capped" flowers. A series of photographs illustrating successive phases in the development of a "capped" flower from the bud stage till the period of full seed development is depicted in Text-figure I, a–f. Just how prevalent this phenomenon is in other species of Grevillea and throughout Protead forms in general is at present unknown, but the author hopes to supply information on this point in the near future.

Possessed of a knowledge of the significance of "capped" flowers in G. Banksii, the writer decided to prosecute a similar investigation in the case of the Silky Oak. A survey of various trees revealed the fact that "capped" flowers, though occurring occasionally, are much more rare than in the species already described. However, specimens were collected in which the corolla persisted long after the perianth segments had been shed from the remaining flowers of the same or adjoining inflorescences. In two selected inflorescences gathered on 3rd November, 1931, one raceme of 65 flowers had 6 "capped" individuals, while in the other with 81 flowers, the number of "capped" specimens was 13, i.e., an average of 14 per cent. This "capped" condition then does occur, though sparingly, and persists beyond the period of normal pollination, and in such cases the increase in size of the ovary clearly points to the fact that in this species also self-pollination and seed-setting do take place. The question then arose as to why the phenomenon is so much more restricted in the case of Grevillea robusta than it is in Grevillea Banksii. Examination revealed that in the former species the perianth segments are much more easily ruptured at the base and apex, and that the repeated contact of numerous birds—large and small—in their search for nectar expedites the rupture and fall of the perianth.

It is only in special cases then—probably where a flower is relatively inaccessible, as at or near the tip of an inflorescence, or where bird visitors for some reason are rare—that the perianth may not be dislodged. The important points that emerge, however, are that should bird visitation fail, then the number of "capped" flowers will be increased, the chances of self-pollination be enhanced, and accordingly a compensating factor operates for the setting of seed.

Further investigations during this season concerning the incidence and significance of "capped" flowers in this species were unfortunately rendered nugatory by the advent of an exceedingly severe windstorm which, by displacing the perianth segments, destroyed all evidence as to "capping" tendencies.

Having demonstrated the structure of the flower about the time of anthesis and pollination, it is natural to transfer one's attention to the subject of pollinating agents.

Observations taken during a clear, sunny day in mid-November, 1930, between the hours of 10 a.m. and 4 p.m., on a tree about 60 ft. high at Wahroonga, some twelve miles from Sydney, revealed the presence of numerous birds of various kinds. These visitors were actively at work sipping the profuse nectar, and at no period of the day was this particular tree devoid of feathered species of some kind.

The birds noticed in greatest number were several species of honeyeaters, and it was noteworthy that one of the less common species, viz., *Meliphaga melanops* (the Yellow-tufted Honey-eater) was present in considerable numbers. Also abundant were *Meliphaga chrysops* (the Yellow-faced Honey-eater) and *Acanthorhynchus tenuirostris* (the Spinebill Honey-eater). In addition, *Anthochaera chrysoptera* (the Little or Brush Wattle Bird) and *Philemon corniculatus* (the Noisy Friar Bird or Leather Head) formed a conspicuous component of the bird population. Other birds were occasionally seen, but those above mentioned were regarded as the forms responsible for the removal of nectar from the flowers of the Silky Oak.

The force exerted by the beak of the bird, while probing for nectar, causes the style to bend forward and downward, thus bringing the tip into contact with some part of the bird's head, which is accordingly dusted with pollen in the case of flowers recently opened, or is brushed over by the stigma in more mature flowers. In this way cross-pollination is readily effected, since a single bird was seen to visit many flowers within a few minutes.

It is worthy of note that the pollination mechanism in *Grevillea Banksii* is much more exact than in *Grevillea robusta*, for in this case the apex of the style and stigma are flattened, and the flower so constructed that the stigmatic disc always comes down on the same spot on top of the visitor's head. This clearly increases the chances of pollination and, taken in conjunction with the close and frequent association of *Meliphaga melanops* with this shrub, would seem to be an important factor in accounting for the relatively high percentage of seed set in this species of *Grevillea*.

In addition to birds, however, the writer constantly observed insects—chiefly bees and ants—in great numbers at work on the flowers of the Silky Oak. These move about at the bases of the flowers, passing from one to another, but, after the freeing of the style and consequent exposure of the pollen, the dimensions of the flower are such that the stigma is too far removed from the nectar to permit of contact between the visiting insect and the stigmatic region, unless perhaps in cases of mere chance. Such visitors, then, are mere nectar-robbers and in no way associated with pollination. On the other hand, birds, on account of their larger size, are pollinating agents, and so *Grevillea robusta* and *Grevillea Banksii* are strictly ornithophilous forms. However, in species of *Grevillea* with smaller flowers, e.g., *Grevillea sericea*, bees were observed to be the pollinating agents.

The infertility of the Proteaceae in general has long been known, and widely commented upon.

H

Within recent years Lawson (1930, p. 374) has drawn attention to the very high percentage of sterile pollen in numerous Protead forms investigated by him, and supplies in tabulated form a list of figures giving actual percentages. Therein *Grevillea robusta* is quoted as having ninety per cent. of sterile pollen, and this fact alone might perhaps satisfactorily account for the relatively low seed production.

Actuated by impressions gained from a study of sections of anthers, which suggested that the figures given by Lawson in the case of *Grevillea robusta* were unduly high, the writer made fresh estimates. Lawson's methods were adopted, although such seem only capable of supplying approximate results. In no case was the percentage of sterile pollen found to exceed 30 per cent., while the figure for *Grevillea Banksii* was in the neighbourhood of 20 per cent. Again, reference to the photograph of the microspores of *Grevillea robusta* accompanying Lawson's paper (Lawson, 1930, Plate x, B) hardly seems to support the high percentage claimed.

Accordingly the writer does not subscribe to the opinion that pollen sterility is the dominating factor in accounting for low seed production, although it is one of the contributing factors. In this connection it is interesting to observe that Hamilton (1931, p. xl) in reviewing Lawson's paper, concludes with the following statement: "I think, therefore, that a good case has been made for the theory that the sterility of the Proteaceae is caused, to a great extent, by the failure of the pollinators to visit flowers, and that it is not due to any inherent infertility."

In the case of *Grevillea robusta*, nectar is produced in such quantity that, on shaking a branch, drops shower down in sufficient quantity to cause unpleasant staining of one's clothes, while handling an inflorescence roughly results in the smearing of the hands.

Nectar is present even before the style is freed, and so at this stage its removal can be of no direct aid in pollination, although the movement of the birds undoubtedly accelerates actual anthesis.

From the facts put forward it would seem, then, that in the case of the Silky Oak, and probably other Protead forms as well, the chief factor controlling seed setting is the visitation of pollinating agents, although the results are modified to some extent by the relatively high percentage of sterile pollen. This latter, however, is to be regarded as a minor factor.

But the matter does not end here; another problem arises: Why is there such a great variation in the amount of seed produced from year to year in Silky Oaks generally, and also in the individual tree? For example, the seed production in 1931 was abnormally high, while the present season's crop for 1932 is exceedingly meagre. Two reasons underlie this phenomenon: first, the exposed position of the pollen, and second, the weather conditions prevailing during anthesis of any one plant, and of Silky Oaks in general.

In 1930, during the period of maximum anthesis and pollen exposure, the climatic conditions were uniformly dry and sunny without acute atmospheric disturbances. Such conditions presented an ideal environment for pollination, and consequently a high seed production was recorded in the early part of 1931.

But during the pollination period of 1931, abnormally dull, cold and wet weather prevailed, and a wind storm of intense violence swept the neighbourhood. In other words, the weather was most unseasonable in regard to low temperatures, high winds, and heavy rains.

Such conditions naturally were antagonistic to pollination on account of general damage to flowers, but more particularly to the displacement of the pollen, which was scattered and lost, and also to the very greatly increased percentage of sterile pollen which had been adversely affected by repeated wetting and drying. During this season, then, pollen sterility was a big factor in accounting for low seed production, but this sterility was due to secondary causes, and not inherent in the original pollen as produced by the flowers.

Different plants open their flowers at different times throughout the general period of anthesis, and this fact, coupled with whether the individual plant occupies an exposed or sheltered position, accounts for the vagaries of seed production from tree to tree, during any particular season.

A résumé of the facts submitted concerning pollination and fertilization of *Grevillea robusta* urges the writer to the conclusion that seed-setting from year to year is controlled by two main factors, the relative abundance of pollination visitors and the meteorological conditions prevailing during the chief period of pollen exposure. If either of these factors is unfavourable the seed production will be low, while if both are inimical to pollination then very few seeds indeed will be produced. On the other hand, if both factors act simultaneously in favour of reproduction, then a maximum seed crop will result.

It is suggested that the conditions controlling seed production in *Grevillea robusta* are of general application in the Proteaceae.

Comparison with Protea Lepidocarpon.

Having obtained a knowledge of the salient features in the life-history of *Grevillea robusta*, one naturally proceeds to effect a comparison with corresponding phases, so far as they are available, in Ballantine's account (1909) of *Protea Lepidocarpon*. Generally speaking the correspondence is very close, and marked agreement is noted in the pendulous nature of the ovule; the single megaspore mother-cell situated amid a group of large cells below the hypodermal layer; the definite meristematic tissue at the base of the nucellus which remains active until time of fertilization; the linear tetrad of megaspores, the chalazal one of which functions; the shape of the embryo sac, and its protrusion, though slight, into the micropyle; the glandular nature of the cells at the tip of the nucellus, and also of the cells of the inner integument surrounding the micropyle; the disappearance of the nucellar tissue soon after fertilization; the absence of a suspensor in, and the globular form of, the embryo and the method of development of the microspores and male gametophyte.

On the other hand, certain contrasting features are to be noted, the most outstanding of which are that the haploid number of chromosomes is only ten, that no irregular protuberances of the nucellus into the integuments are present, that the origin of the ovules is in the middle region of the ovarian cavity, and that the embryo sac does not become densely packed with starch, although starch grains do aggregate in quantity in the neighbourhood of the ovum just prior to fertilization.

These dissimilarities, however, do not to any significant extent detract from the essential agreement of the phases available for comparison, or from the validity of the deduction that a very close affinity exists between the two genera under comparison.

Conclusions.

A study of the floral organogeny shows that the perianth is essentially primitive, consisting, as it does, of four free segments arising from the rim of

a very slightly concave torus, which in turn is slightly inclined to the plane at right angles to the axis of the flower. There is no evidence that there ever has been any differentiation into a calyx and a corolla, or that the flower represents a reduced type.

The flower has bilateral symmetry, the plane being the antero-posterior one. Zygomorphy is induced by the later development of the style, ovary and ovules being accentuated in the plane referred to. The early fusion of the meristematic zones, which give rise to the perianth segments and stamens respectively, is a feature of advance, but one easily effected. Moreover, the degree of fusion varies throughout the Family. In fine, the facts of organogeny relative to the perianth and androecium indicate an essentially primitive structure for the flower in question.

The development of the anther is characterized by the production of a thick wall, a fibrous layer of hypodermal origin, a plate one cell thick of spore-mother-cells, a normal tapetum, and a somewhat unusual, but none the less simple, method of dehiscence.

The carpel shows very clearly its single, foliar origin, and bears two ovules with marginal placentation. These ovules are anatropous, but as anatropy is a feature of early evolutionary attainment, the gynoecium as a whole, and the resulting fruit, are essentially primitive in structure. Simplicity in fruit structure is characteristic of the family.

Pollination in the Proteaceae is effected either by insects or birds, and is also associated with self-pollination, but the mechanisms involved are not of a type embodying any high degree of specialization in floral structure.

Again, an appreciation of the salient features in the life-history of *Grevillea robusta* brings a realization of the marked simplicity of the type chosen for this study. Further, as already explained, a survey of the outstanding phases in the life-histories of *Grevillea robusta* and *Protea Lepidocarpon* results in the realization that a very close agreement exists between the two forms, but the chief interest of such a comparison is centred in the fact that certain peculiarities in *Protea Lepidocarpon*, such as the glandular cells limiting the apex of the nucellus, the similar cells associated with the micropyle, the suggestion of a former multicellular sporogenous tissue in the ovule, and the rounded embryo devoid of a suspensor, are all represented in *Grevillea robusta*.

The obvious deduction is that such peculiarities could not have been evolved independently, but have been inherited from a common ancestry. Now, present land distribution does not permit of direct contact between Africa and Australia; moreover, the separation of these two continents seems to have persisted since Carboniferous times when Gondwanaland connected Australia, India and South Africa (Berry, 1918).

Again, Australia is considered to have been joined with the Asiatic mainland at least during the upper Cretaceous period (Benson, 1923, pp. 50–52), but probably a complete separation has existed since the beginning of the Eocene. From such facts one may infer that the ancestors of the Proteaceae—as well as those of other Australian Families—migrated southwards from an Asiatic centre of distribution into Australasia and Africa, as then constituted, during later Cretaceous times. It may be stated that an intensive study of the Epacridaceae by the writer fully supports the above contention.

Keeping in mind the general characters of the Family as a whole, and of the details regarding *Grevillea robusta* made available as a result of this

investigation, and of *Protea Lepidocarpon* as described by Ballantine, one may proceed to a consideration of the affinities of the Proteaceae and the real position of this Family in a natural scheme of classification. As already pointed out in the early pages of this publication, different writers have claimed close kinship for the Proteaceae with the Santalales, Thymeleaceae, and Rosales respectively.

At this juncture the writer believes that little purpose would be served in recounting and reconsidering the minutiae of detail pertaining to gross morphology. This type of evidence has already been marshalled and dealt with by eminent systematists, and with the results indicated. Further progress is rather to be achieved by comparing details of the life histories—so far as they are available—of the forms most intimately concerned with the purpose in view, gross characters only being used as a check on deductions made from other evidence.

Dealing with the Santalales claim, it may be said that the evidence from gross morphology is not of the most convincing nature. If any real affinity does exist, then the modifications which have taken place in the various Santalalean Families since the common connecting type was departed from, are so great that features indicative of any real kinship have become obscure, almost to the point of obliteration.

Turning to the evidence supplied from investigations relative to life-histories, much information has been supplied by Guignard (1885), but comparison with corresponding phases in *Grevillea robusta* fails to substantiate any claim for close kinship. Again, in more recent times, Skottsberg (1913) has investigated the morphology and embryology of the Myzodendraceae, but here again the results are negative, for although the embryology runs parallel with that of the early and medium stages in *Grevillea robusta*, there is nothing beyond that on which any near relationship may be based.

Turning now to the claim for a Proteales-Thymeliales affinity, it may be conceded that, despite considerable variation in floral structure within the Families of the Thymeliales (Hutchinson, pp. 150–152) there is nothing in gross morphology really incompatible in such an association. A nice series in the gradual fusion of the perianth segments is illustrated in passing from the Proteaceae where fusion is suggested, to the Geissolomataceae where the "calyx" is shortly tubular and four-partite almost to the base, and thence to the Thymeleaceae and Penaeaceae where the "calyx" is markedly tubular. Throughout, the stamens are epiphyllous and the ovary superior. Accordingly a more searching scrutiny may be the more hopefully instigated. An approach to the problem may best be made by reference to the investigations of Stephens on the Penaeaceae (1908 and 1909) and also on *Geissoloma marginatum* (1909a). Dealing first with the Penaeaceae, it is to be noted that in the three genera examined, viz., *Sarcocolla*, *Penaea* and *Brachysiphon*, the development of the female gametophyte is abnormal, Stephens (1909c, p. 381) pointing out that "the first two divisions form a tetrad of nuclei arranged just as in *Peperomia*: these separate and each divides again twice, so that four groups of four nuclei each are formed at the periphery of the sac. Cells are organised round three of the nuclei in each group, in the form of an egg-apparatus."

There is, of course, nothing like this in *Grevillea robusta*, and so far there is no general agreement as to the significance of, or cause underlying, the abnormal behaviour noted in the Penaeaceae and *Peperomia*. Further, Stephens

(1909, p. 370) says: "As regards the other Orders with which the Penaeaceae are grouped, the Thymeleaceae is the only one of whose life-history any account has been published, Strasburger having recently investigated species of *Wiksstroemia, Daphne* and *Gnidea*, none of which show a similar departure from the normal."

The embryology of the Penaeaceae conforms closely with that of *Geissoloma marginatum* (Stephens, 1909a), but it would not be valid to establish any connection on that feature alone. Accordingly, the Penaeaceae, if retained within the Order Thymeliales, must be regarded as occupying an isolated position, and with its kinship non proven.

However, on comparing *Geissoloma marginatum* (Stephens, 1909a) with *Grevillea robusta* marked similarities are evident in the general structure of the nucellus, the normal development of the functional megaspore and female gametophyte, the embryo—in the early stages oval, and later becoming round—the emergence of the cotyledons amid a greatly elongated copious endosperm, which in turn lies in a sap-containing cavity due presumably to the collapse of the nucellus. Accordingly, the evidence justifies one in recognizing an affinity between the Proteaceae and the Geissolomataceae without admitting any close connection between these two Families and the Penaeaceae. At such a stage one feels how unfortunate it is that the investigations referred to were not of a wider scope in the individual, so that other points of contact might be sought.

Finally, there remains the claim that the Proteales may best be associated with the Rosales. The amount of ground which would of necessity be covered in making a critical examination of such a claim would be so extensive as to extend impracticably the scope of this section of the present investigation. Moreover, amid the plexus of forms included in this Order there is no obvious section to which the inquiry might be confined, and more important still is the fact that the writer is convinced that much more research must necessarily be effected in the realm of adjoining and comparatively unknown Orders and Families before such a task could be attempted with any hope of success commensurate with the labour involved.

Suffice it to say in conclusion that, while the results of this research on *Grevillea robusta* are not incompatible with a real affinity between the Proteaceae and the Geissolomataceae and through them with the Thymeleaceae in general, still the view must not be lost sight of that the simplicity of the Proteaceae may well be primitive, in which case an affinity with some Order much lower in the scale of evolution than the Thymeliales will have to be sought. The success of such a quest will probably depend on the extension of such investigations as the one now completed.

SUMMARY.

Floral Organogeny.—The primordia of the individual members of the various sets of floral organs arise separately, and in acropetal succession. The torus is very slightly concave and from its rim originate the four separate primordia of the perianth segments, which later simulate gamophylly owing to the interlocking of the contiguous marginal cells. There is no indication that a separate calyx and corolla ever existed. The stamens arise as individuals from the torus, but become epiphyllous at a very early stage. A single carpel occupies the organic apex of the floral receptacle, and soon after its inception, a crescent shaped nectarscale arises between the gynoecium and the perianth segments, and on the adaxial side of the flower.

Microsporangium.—The archesporium consists of a plate one cell thick which divides by periclinal walls giving rise to a primary parietal and a primary sporogenous layer respectively. The cells of the former divide repeatedly, thereby producing a wall four cells thick lined internally by a tapetum forming a fifth layer. The sporogenous cells, without further division, function as the spore mother-cells, which undergo reduction division, whereby pollen tetrads are produced. At dehiscence the microspores are binucleate, and tetrahedral in shape, with a pore at each of the four corners. The exine is very thick and heavily cutinized. The fibrous layer is hypodermal, and dehiscence is effected by the orderly separation without rupture of the overlapping margins of adjacent microsporangia—a somewhat rare mechanism in Angiosperms.

The male gametophyte.—The mature microspores contain a small generative and a larger vegetative nucleus separated by a thin membrane. Just prior to germination vacuolation becomes evident, and a single pollen tube emerges at one of the four unthickened areas of the pollen grain. Germinating grains were seen on the stigma, and the pollen tube was traced in its growth down through the style, and along the wall of the ovarian cavity, whence it crossed to the ovule near the apex and, growing along its surface for a short distance, eventually entered the micropyle, which is lined by cells of a glandular nature.

Nectar secretion.—The morphological nature of the nectar-scale is explained, and profuse nectar secretion from this organ, and also from the cells of the inner epidermal layer of the basal regions of the perianth segments, is described.

Ovule.—The two nucellar primordia arise from opposed margins of the carpel, and in the region adjoining the base of the ovarian cavity.

A marked feature is that development of the ovule is almost entirely due to the pronounced activity of a meristematic zone of cells in the basal region of the nucellus.

An inner and an outer integument arise relatively late and develop slowly, but eventually enclose the nucellus except in the region of the micropyle. The nucellus is of the massive type, and eventually a single deep-seated megaspore mother-cell is differentiated. Contiguous cells show sporogenous tendencies, but never develop into spore mother-cells. A linear tetrad is produced, the innermost megaspore of which enlarges and destroys the others.

The female gametophyte.—The functional megaspore initiates germination by vacuolation and an increase in size. This is succeeded by the bi-nucleate, four-nucleate and eight-nucleate stages respectively of the embryo sac. The egg apparatus, polar nuclei and antipodal cells are normal in structure and polarity. During this development only part of the encasing nucellar tissue is invaded, except at the micropylar end, where destruction of the nucellus is practically complete.

Fertilization.—The available evidence indicates that one male nucleus fertilizes the oosphere, while the other joins the two polar nuclei which were seen to fuse in close proximity to the ovum.

Endosperm.—The endosperm nucleus divides before that of the oospore which passes to, and impinges upon, the micropylar end of the sac. Numerous free endosperm nuclei are formed, but few of these are located at the chalazal end of the sac. Wall formation in the upper and central regions accompanied by a rapid extension in length of the embryo sac then supervenes. The growth of the endosperm tissue is relatively slow, and barely fills the antipodal end of

the large sac. Enzyme action brings into solution, and completely destroys, the remaining nucellar tissue.

Antipodal Cells.—These remain quiescent, and have no apparent functional significance.

Embryology.—Development of the oospore begins relatively late, but once started proceeds with great vigour. There is no suspensor. In its early stages the embryo is roughly oval in shape, and flattened in the antero-posterior plane. Later it becomes round and disc-like in form and subsequently dermatogen, periblem, plerome, cotyledons, plumule and radicle are differentiated. Each cotyledon has a peculiar double lobe at its base. The endosperm is eventually destroyed.

Pollination.—Careful attention was given to this problem. The flower is markedly protandrous, and transfer of pollen is effected by various birds, the names of which are specified. The pollination mechanism is described. Many visiting insects are mere nectar-robbers. Self-pollination is possible and does occasionally occur in *Grevillea robusta.* In *Grevillea Banksii,* however, thirty to forty per cent. of the flowers may be self-pollinated. The phenomenon of self-pollination may be widespread throughout the Proteaceae.

Seed structure.—The mature seed is non-endospermic and bears a broad wing which is morphologically an extension of the outer integument.

Seed production.—The reasons underlying the variation in the heaviness of the seed crop from year to year, and among individual trees, are given. Factors responsible for the notoriously low seed production in the Family as a whole are described.

Comparisons and affinities.—Comparison with the South African form *Protea Lepidocarpon* is made, the genetic relationships of the Proteaceae are discussed, and some general deductions regarding plant distribution and area of origin of the Family are recorded.

Acknowledgments.

List of References.

BALLANTINE, A. J., 1909.—Preliminary Note on the Embryo-sac of *Protea Lepidocarpon.* *Ann. Bot.,* xxii, pp. 161-162.

BENTHAM, J., 1870.—Flora Australiensis.

BENSON, W. N., 1923.—Palaeozoic and Mesozoic Seas in Australasia. *Trans. New Zealand Institute,* 54, pp. 1-62.

BERRY, E. W., 1918.—Annual Report of the Smithsonian Institution.

BEWS, J. W., 1927.—*New Phytologist,* xxvi, Nos. 1 and 2.

BROUGH, P., 1923.—Preliminary Note on the Embryo Sac of *Styphelia longifolia.* PROC. LINN. SOC. N.S.W., xlviii, Pt. iv.

————, 1924.—Studies in the Epacridaceae. I. The Life-history of *Styphelia longifolia.* PROC. LINN. SOC. N.S.W., xlix, Pt. ii.

————, 1927.—Studies in the Goodeniaceae. I. The Life-history of *Dampiera stricta.* PROC. LINN. SOC. N.S.W., lii, Pt. iv.

CAMPBELL, D. H., 1897.—A morphological Study of *Naias* and *Zannichellia.* *Proc. California Acad. Sci.*

FRANCIS, W. D., 1929.—Australian Rain-Forest Trees.

GUIGNARD, L., 1885.—Observations sur les Santalacées. *Ann. Sci. Nat.,* Bot. vii, 2, pp. 181-202.

1.—*Grevillea robusta.*   2.—*G. Banksii.*